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HISTOLOGICAL CHARACTERISTICS OF PLANTS GROWN IN TOXIC CONCENTRATIONS OF BORON[1]

By IRMA E. WEBBER

Collaborator, Division of Western Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Although small amounts of boron are considered essential for the normal growth and development of many widely different species of the higher green plants (*7, 8, 15, 16, 17*),[2] excessive concentrations of this element have long been known to be toxic (*11*). Injury resulting from boron occurring in excessive concentrations as a natural constituent of irrigation waters is a factor of agricultural importance in certain areas in tne western United States (*5, 9, 13*). The wide variability of optimum and maximum concentrations of boron for the growth of different species and the characteristic external symptoms of boron injury exhibited by numerous plants have been discussed recently by Eaton (*5*). Generally, leaf injury is the characteristic manifestation of boron toxicity, the older leaves showing the most pronounced symptoms. Briefly, the common symptoms of boron injury to leaves are the yellowing of tips and margins (often followed by marginal or spotted browning, which may be followed by premature defoliation) and the subsequent production of malformed leaves. Under high-boron conditions stone-fruit trees seldom exhibit these leaf symptoms, but the petioles and larger veins of the leaves of prune and apricot trees may become brown and rough and occasionally exude gum. The twigs frequently exude gum, especially above the leaf and twig insertions, and die at the tips; their internodes may be much shortened, and their nodes are often enlarged.

MATERIAL AND METHODS

The histology of plant organs showing boron injury has hitherto received but scant attention. The specimens upon which the present study is based were mostly taken from the cultures of this Division at the Rubidoux Laboratory, Riverside, Calif. The boron-injured prune specimens were from a plant grown in a sand culture supplied with a culture solution containing 9 parts per million of boron. The boron-injured peach, apricot, and grape were supplied with a solution containing 6 p. p. m. of boron; and the boron-injured lemon was supplied with a solution containing 3 p. p. m. of boron. These specimens were studied in comparison with healthy specimens of the same age and variety grown under similar conditions except for lower concentrations of boron in the culture solution. In the case of lemon and grape, the healthy specimens received 1 p. p. m. of boron in the culture solution. Specimens of healthy peach, prune, and apricot

[1] Received for publication Nov. 12, 1934; issued April 1935.

[2] Reference is made by number (italic) to Literature Cited, p. 193.

were grown in a culture solution to which no boron had been added. The boron contained in these solutions was generally less than 0.1 p. p. m., occurring as impurities in the c. p. chemicals or in the quartz sand. Specimens of field-grown prunes and apricots supplied with irrigation water of high boron content and comparable specimens from adjacent orchards irrigated with water of low boron content were also examined. The material was sectioned fresh or after killing and fixing in formalin-acetic-alcohol, and mounted unstained in glycerin.

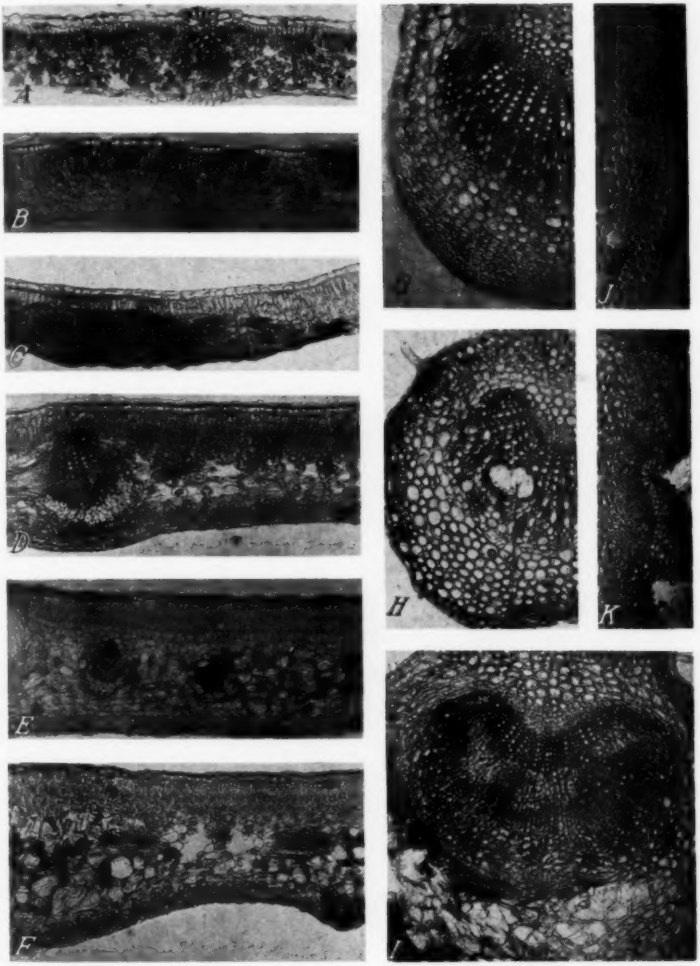
ABNORMALITIES OF LEAVES

A histological study of boron-injured leaves of lemon, grape, prune, and apricot showed that their structural abnormalities occur chiefly at points showing macroscopically visible injury. The yellowish areas which commonly precede the brown margins of boron-injured lemon leaves and the pale-green areas which often border the brown spots of boron-injured grape leaves are attributable to the conversion of chloroplasts into leucoplasts in such areas. Except for the reduced number of chloroplasts, the mesophyll of injured leaves shows no histological differences from that of healthy leaves at these points (pl. 1, *A–F*).

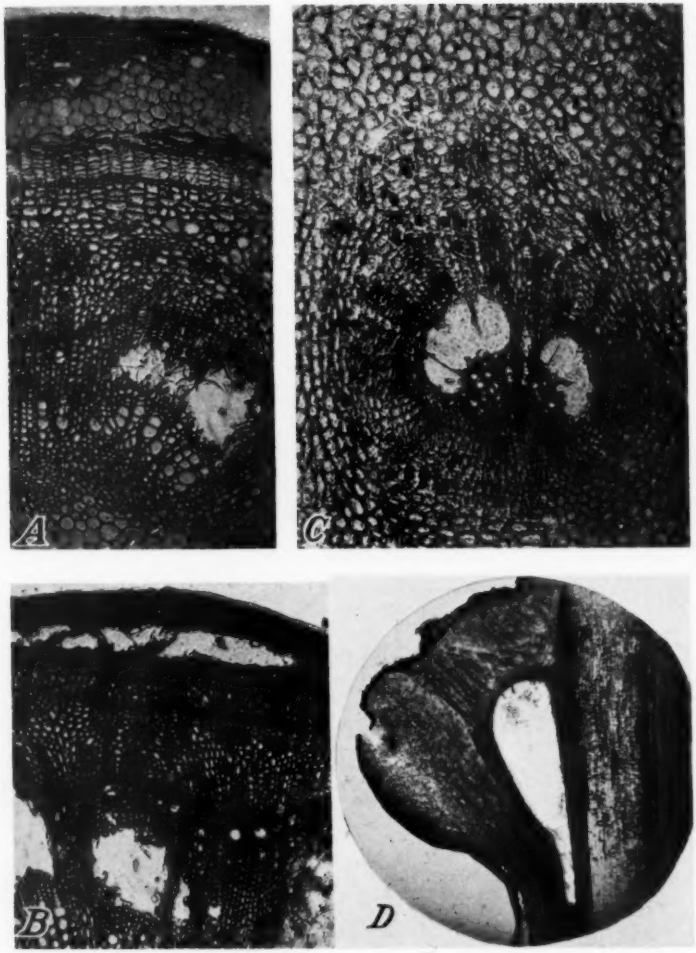
Cells of normal size and arrangement, showing various stages in the disorganization of their protoplasts, are visible in the discolored areas present at or near the margins of boron-injured lemon and grape leaves and in the larger veins and petioles of boron-affected grape, apricot, and prune leaves (pl. 1, *B*, *C*, *E*, *H*). In the early stages of this protoplasmic disintegration, the browned protoplasts completely fill the cell lumina, but later they often shrink away from the cell walls. Such browning of cells is at times observable in the epidermis, palisade parenchyma, spongy parenchyma, and parenchyma of the larger veins, but usually does not extend from upper epidermis to lower epidermis at a given point in the lamina. The conducting tissues of veinlets surrounded by necrotic mesophyll are frequently filled with brownish contents of gummy appearance.

Associated with such necrotic areas, very limited hypertrophied and hyperplastic areas are occasionally visible in the mesophyll of boron-injured lemon leaves (pl. 1, *F*) and in the petioles and midribs of boron-injured prune (pl. 1, *I*) and apricot leaves. At times a phellogen layer develops beneath the necrotic parenchyma of apricot and prune petioles (pl. 1, *K*) and midribs, resulting in the sloughing off of small, macroscopically visible scales of dead tissue. In prune petioles and midribs with externally visible injuries, gum pockets formed by disintegration of xylem elements may be present (pl. 1, *H*). The absence of changes in the vascular anatomy of boron-injured citrus leaves is reported by Haas (*6*).

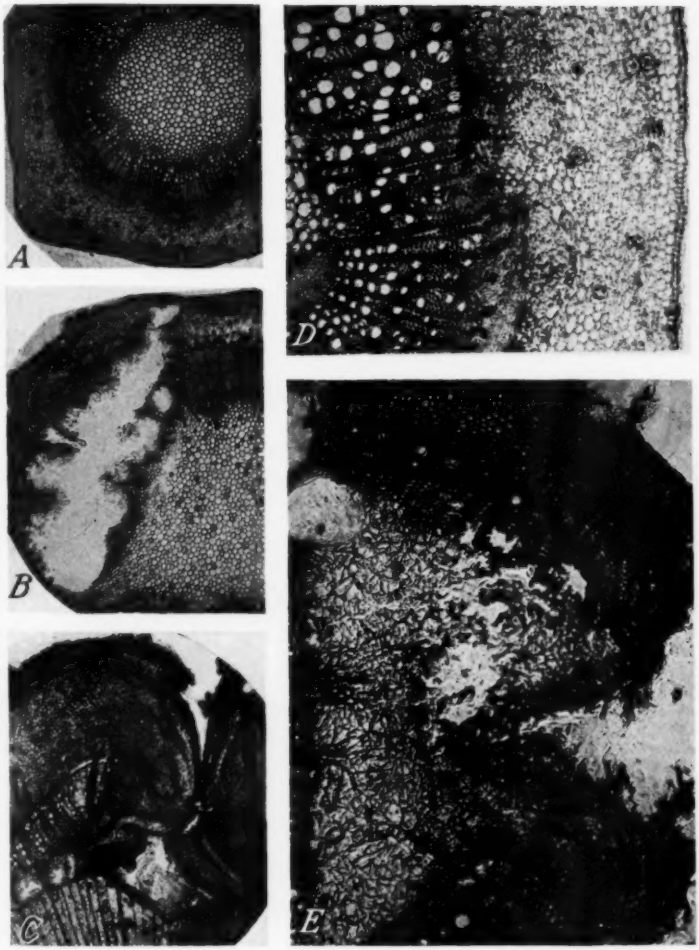
The only additional way in which boron-injured leaves were observed to differ histologically from comparable healthy leaves is in the presence of fewer calcium oxalate crystals in the injured leaves. This difference is most noticeable in the phloem of petioles and veins of apricot and prune leaves (pl. 1, *G*, *H*). In this connection it is noteworthy that Haas (*6*) reports that chemical analyses have shown that boron-injured lemon and walnut leaves contain a lower percentage of calcium and a higher percentage of potassium than healthy leaves of these species.



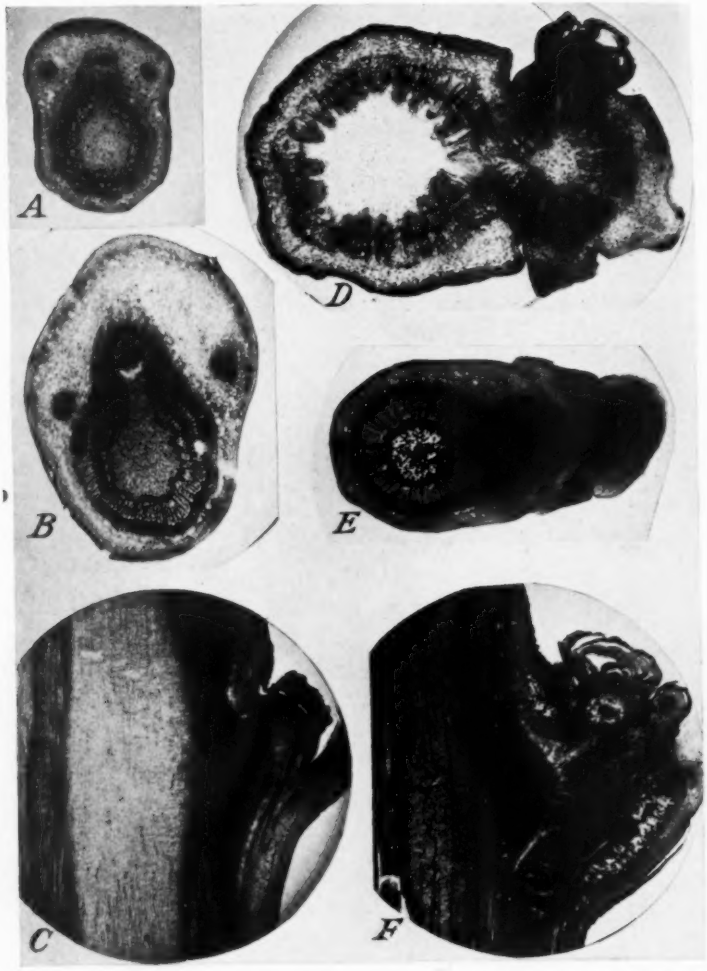
A–F, Cross sections of leaf blades: *A*, Healthy grape; *B–C*, boron-injured grape; *D*, healthy lemon; *E–F*, boron-injured lemon. × 50. *G–I*, Cross sections of prune midribs: *G*, Healthy; *H–I*, boron-injured. × 35. *J–K*, Cross sections of prune petioles: *J*, Healthy; *K*, boron-injured. × 35.



A–B, Cross sections of internodes of current season's stems: *A*, Boron-injured prune; *B*, boron-injured peach. × 79. *C*, Cross section of branch trace in node of boron-injured prune. × 79. *D*, Longitudinal section of node of boron-injured prune. × 9.



A–B, Cross sections of internodes of current season's stems of apricot: *A*, Healthy; *B*, boron-injured × 9. *C*, Cross section of internode of 2-year-old stem of boron-injured prune. × 9. *D–E*, Cross sections of internodes of current season's stems of peach: *D*, Healthy; *E*, boron-injured. × 79.



A–B, Cross sections of nodes of current season's stems of apricot: *A*, Healthy; *B*, boron-injured. × 7.5. *C*, Longitudinal section of node of healthy apricot. × 7.5. *D–E*, Cross sections of nodes of current season's stems of prunes: *D*, Boron-injured; *E*, healthy. × 7.5. *F*, Longitudinal section of node of boron-injured apricot. × 7.5.

ABNORMALITIES OF STEMS

The greatest abnormalities in stem structure of boron-injured stone-fruit trees are to be found in shoots that do not live beyond the first year, although enlarged nodes are often conspicuous on 2- and 3-year-old branches. In current season's stems of boron-affected prune, peach, and apricot trees, necrotic areas comparable to those discussed in connection with boron-injured leaves occur in the epidermis and subjacent cortical parenchyma (pl. 2, *A*). As in the case of petioles of these species, phellogen (pl. 2, *A*) may develop beneath the necrotic tissues which at times slough off in the form of small scales. Since the necrotic areas of cortical parenchyma are commonly lenticular in cross section and may extend deeply into the cortex, such phellogen formation is in contrast to that normally occurring in the subepidermal layer of cortical parenchyma cells. As in the leaves, the current season's stems of boron-injured stone-fruit trees, particularly those of peach and apricot, may contain noticeably fewer calcium oxalate crystals than comparable healthy specimens (pl. 3, *A*, *B*, *D*, *E*). This difference in calcium oxalate content is visible in cortex, phloem, and pith.

Small lysigenous cavities filled with gum are frequently present in the cortex of first-year stems of boron-injured stone-fruit trees (pl. 2, *B;* pl. 3, *B*). The pressure exerted by the gum in these cavities is often sufficient to rupture the epidermis, but at times merely distends the overlying tissues and causes minute swellings on the periphery of the stem. Although such cortical gum cavities are rather numerous, axial lysigenous gum ducts in the xylem are far more abundant and conspicuous (pl. 2; pl. 3, *B*, *C*, *E*). The gum ducts are apparently formed by the disintegration of abnormal xylem parenchyma. Such tissue at times persists and may form a large percentage of the wood normally composed of other axially elongated xylem elements (pl. 3, *E*). In the internodes the gum ducts in the xylem may encircle the stem. At the nodes, xylem elements of leaf and branch traces, as well as those of the main vascular cylinder, may disintegrate to form gum ducts (pl. 2, *C*). Occasionally cortical gum cavities and gum ducts in the xylem become united through the disintegration of intervening cambium, phloem, and cortical elements (pl. 3, *B*, *C*). Such fusion of gum cavities indicates that a large proportion of the gum which accumulates on the surface of these stems has its origin in the xylem. Gum pockets may also occur in the pith immediately beneath the dead portion of twigs which have died at the tips. Gum ducts similar to those occurring in first-year stems may also be formed in later years (pl. 3, *C*).

The enlargement of nodes of boron-injured stone-fruit trees (pl. 2, *D;* pl. 4, *B*, *D*, *F*) is chiefly due to the growth of axillary and accessory buds which would normally remain dormant (pl. 4, *C–F*). The branch traces of such nodes are therefore usually considerably larger than those in healthy stems. More or less hyperplasia commonly occurs in the cortex of the enlarged nodes (pl. 2, *D;* pl. 4, *A*, *B*), and hypertrophy of some of the parenchyma cells is not unusual. In the shortened internodes of boron-injured stone-fruit stems the cortical parenchyma cells remain shorter and exhibit less variation in size than similar cells in comparable healthy stems.

DISCUSSION

From the foregoing it is evident that the manifestations of boron toxicity which are characteristic of some plants are lacking in others. Butler (*2*) has cited numerous instances in which similar reactions of tissues may be brought about by widely different stimuli. Hence it is not surprising to find that the histological evidences of injury by boron are, in general, similar to those attributable to other causes. The enlarged nodes of boron-injured stone-fruit trees are not unlike those of French prune twigs affected with exanthema (*14*). Lysigenous gum ducts in stone-fruit trees have been induced by a wide variety of stimuli including a number of fungi, bacteria, insects, chemical substances, and traumatisms (*3*). Priestley and Woffenden (*12*) have shown that phellogen formation in both normal and abnormal positions results from a set of conditions that may be fulfilled in many ways. The calcium oxalate content of the aboveground organs of plants is influenced by various factors which influence the rate of transpiration (*10*), as well as by the composition of the nutrient solution (*1*). Hypertrophied cells and hyperplastic areas in leaves may result from such diversified stimuli as fungi, insects, injurious vapors (*2*), and boron deficiency (*7*). Chloroplasts frequently change back into leucoplasts in starving cells and in those incited to abnormal growth (*10*). Localized browning of cells may be initiated by sunburn (*4*), wounding (*10*), or virus infection.

The cases cited above are sufficient to indicate that a toxic concentration of boron in the nutrient solution cannot be regarded as a stimulus to a specific reaction reflected as a specific histological change, but that the histological changes induced by excessive concentrations of boron are correlated with the inherent capacity of a species to respond to stimuli. Theoretically every living plant cell possesses the potential capacity to react to various stimuli by hypertrophy, hyperplasy, or the development of meristematic tissues (*2*, *10*), but the potentiality is greater in certain tissues than in others, and these tissues show greater potentiality in certain species than in others. It has been suggested that one of the roles which boron plays in the plant is that of stimulating meristematic activity (*1*, *7*, *8*, *16*, *17*). Structural abnormalities resulting from stimulation to abnormal growth are met with in the stems, petioles, and veins of some boron-injured stone-fruit trees and to a very limited extent in boron-injured lemon leaves. Such of these structural abnormalities as occur in the cortex of stems, the parenchyma of petioles and veins, and the mesophyll of leaves are commonly associated with necrotic areas in which the cells are of normal size and arrangement. Similar necrotic areas are found in the mesophyll of boron-injured lemon and grape leaves which show no structural abnormalities. These necrotic areas are commonly preceded and bordered by areas in which partial degeneration of chloroplasts indicates a weakened condition of the protoplast.

Kelley and Brown (*9*) and subsequently Scofield and Wilcox (*13*) have shown that boron accumulates in the leaves of boron-injured citrus and walnuts. Eaton (*5*) has shown further that the tissues manifesting the greatest injury are those in which boron has accumulated in relatively high concentrations. It seems probable, therefore, that an excessive concentration of boron in the cell often injures the

protoplast to such an extent that it undergoes a progressive degeneration first affecting the chloroplasts. However, in some instances an excess of boron may stimulate the protoplast to abnormal growth or division.

SUMMARY

Injury to plants resulting from boron occurring in excessive concentrations as a natural constituent of irrigation waters is a factor of agricultural importance in certain areas in the western United States.

The macroscopic symptoms of boron injury to various plants have been described in previous papers.

Histological characteristics of boron-injured lemon, grape, prune, and apricot leaves, and prune, peach, and apricot stems are described herein.

In general, the plant parts manifesting the greatest injury are those in which boron has accumulated in relatively high concentrations.

Both macroscopic and microscopic manifestations of boron toxicity which are characteristic of some plants are lacking in others. This indicates that a toxic concentration of boron in the nutrient solution is not a stimulus to a specific reaction reflected as a specific histological change.

The histological evidences of injury by boron are, in general, similar to those attributable to other causes. This suggests that the observed abnormalities are correlated with the inherent capacity of a species to respond to stimuli.

It seems probable that an excessive concentration of boron in the cell often injures the protoplast to such an extent that it undergoes a progressive degeneration. Chloroplasts, when present, are affected first.

In some instances an excess of boron may stimulate the protoplast to abnormal growth or division.

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EFFECT OF VARIATIONS IN STAND ON YIELD AND QUALITY OF SUGAR BEETS GROWN UNDER IRRIGATION [1]

By H. E. BREWBAKER, *associate agronomist*, and G. W. DEMING, *assistant agronomist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

In agronomic experiments, variations in stand between treatments or varieties are commonly encountered and must be taken into consideration at harvest. It is essential to know the growth response to such variations in order that the relative importance of any error resulting from them may be determined. This is true particularly for such crops as corn or sugar beets, which are either planted in hills or thinned to individual plants spaced at designated intervals in the row.

In this connection, certain published studies are of interest.

De Haan and Klijnhout (*2*) [3] studied the relationship between density of stand and yield of sugar beets. They found a gradual decrease in weight per beet and a corresponding increase in total weight per unit area as the space allotment per plant decreased, the number of beets per acre varying from 16,000 to 45,000 while the distance between the rows was held constant at 40 cm. The studies were conducted on three fields of different degrees of fertility, the results being similar for all three.

Souček (*6*), in a study of the relation between stand and yield, in rows 42 cm apart, found a direct and positive correlation to exist within the observed limits of 11 to 38 beets per unit of row 8 m in length.

Willcox (*7*) reviewed the work of De Haan and Klijnhout (*2*) and Souček (*6*) and interpreted their results in relation to the Mitscherlich effect law of growth factors.

Munerati, Mezzadroli, and Zapparoli (*4*) found no constant relation between the available space and weight per beet. Increased space available was found to be only partially compensated for by the increased weight of some of the beets.

Pack (*5*) obtained the correlation values 0.20 ± 0.03 for relative space with weight per beet and 0.21 ± 0.03 for space with sucrose content or total weight of sucrose per beet. He found no correlation, however, between space and percentage of sucrose.

Immer (*3*) studied individual variability of sugar beets grown without irrigation. He found weight of root to be much more variable than percentage of sucrose, and the regression of sucrose percentage on weight to be not entirely linear. The effect of variations in stand

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[2] The writers wish to express their appreciation to A. W. Skuderna, principal agronomist; G. H. Coons, principal pathologist; and F. R. Immer, associate geneticist, Division of Sugar Plant Investigations, for helpful suggestions in connection with the preparation of the manuscript and the presentation of the data. Acknowledgment is made to E. G. Barlow, a farmer near Fort Collins, who made a portion of his beet field available for experimental studies, and to the American Beet Sugar Co. for land and facilities at Rocky Ford, Colo.

[3] Reference is made by number (italic) to Literature Cited, p. 210.

were largely eliminated by removing before harvest the beets adjacent to noticeable skips.

It was the object of the studies reported in this paper to determine the extent to which the sugar beet will respond to additional space allotment per plant under irrigation in Colorado and to obtain a measure of the extent of the possible error which may be introduced into agronomic experiments as a result of variations in stand.

MATERIALS AND METHODS

Studies were conducted in 1930 on 2 irrigated beet fields near Fort Collins, Colo., and in 1931 on 2 near Fort Collins and 1 near Rocky Ford, Colo.

The results for 1930 are presented for the Barlow farm and the College farm near Fort Collins. The same commercial beet variety was used for both fields. With the exception of four comparisons made on the College farm, the rows were 20 inches apart, and the beets were spaced 12 inches in the row, the blocking having been done mechanically.

On the Barlow farm, all beets from a plot 22 rows wide by 132 feet long were harvested individually, and a record was made of the location of each beet with reference to blank spaces in the row and in the adjacent rows. Each beet was numbered and sampled with a rasp and analyzed for sucrose. The average weight and percentage of sucrose were computed for each lot having a particular type of competition, and comparisons were made with the check or "normally competitive" [4] beets on the basis of the standard-error values (experiment A in tables 1 and 2). A nearby portion of the same field was divided into 1,600 single-row plots 16 feet long (experiment A–1 to A–5 in table 6). The total number and the weight of beets were recorded for each plot, the data being used to determine the regression of weight of beets on stand. The type of competition was also recorded for every plant harvested. The relative response in beet weight to increased space allotment was determined from the portion of the same field harvested individually as described above. It was then possible to adjust the total weight of each plot having an imperfect stand to an expected value based on a perfect stand.

The regression of weight of beets on stand was also obtained for a uniformity study conducted in 1931 (experiment E, table 6), in which 1,280 plots were harvested in a manner similar to that described above for the 1,600 plots in 1930, except that no record was taken of the type of competition for individual beets.

On College farm in 1930 (experiment B in tables 1 and 3), the data were obtained entirely from paired comparisons. A "test" beet with a certain specific relation to one or more blank spaces in the same row or in adjacent rows was paired with the nearest check beet having competition on all four sides. So far as possible, the check beet always occupied the same relative position with reference to the test beet. The percentage sucrose determinations were made on bulked lots of beets representing each test-and-check comparison.

In 1931 there were 5 or 6 replications of each treatment at Fort Collins (experiment C in tables 1 and 4) and at Rocky Ford (experiment D in tables 1 and 5). Each "block" included several treat-

[4] As used in this paper, "normally competitive" beets have competition from beets adjacent on 4 sides, while "noncompetitive "beets lack competition on 1 or more sides.

ments, the blocks being distributed at random in each of the replications.[5] The Fort Collins field was planted by hand, with rows 20 inches apart and the beets spaced 12 inches apart in the row, the desired adjustments in stand being made at the time of thinning according to the plan shown in figure 1. At the Rocky Ford station the planting was done by machine; the width of row and spacing were the same as at Fort Collins, the accuracy of spacing being assured through the use of a marked tape.

The type of competition for the beets harvested in the various rows shown in figure 1 was as follows:

Row	*Type of competition*
A	Adjacent to one blank in adjacent row
A	Adjacent to two corner blanks in adjacent row
B	Between two blanks in same row
C	Between two blanks in adjacent rows
C	With four corner blanks
D	Check (singles surrounded by singles)
E	Blanks on four sides
F	Blanks on three sides
G	Adjacent to single blank in same row
H	Adjacent to single corner blank
I	Doubles surrounded by singles
I	Singles adjacent to doubles in same row and to singles in adjacent rows
J	Triples surrounded by doubles
J	Doubles adjacent to triples in same row and to doubles in adjacent rows

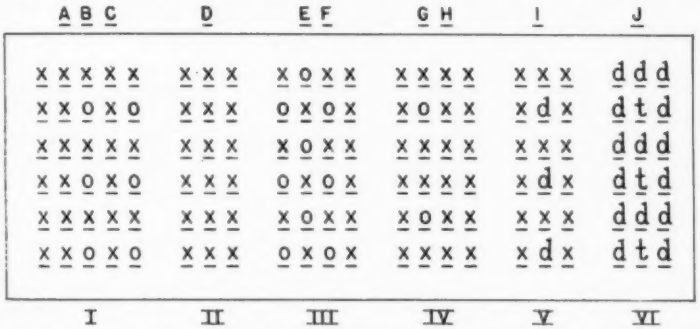

FIGURE 1.—Diagram of plan used for the competition study in 1931: I–VI, Blocks; *A–J*, rows; *x*, single plant; *o*, blank hill; *d*, double, i. e., 2 plants in one hill; *t*, triple, i. e., 3 plants in one hill.

The fertilizer experiment in 1931 (experiment F, table 6), data from which were used in calculating regression of weight of beets on stand, consisted of a randomized distribution of treatments and check plots. The original stand was not very satisfactory and the thinning labor was directed to leave as good a stand as possible. As a result there was a wide variation in spacing and uniformity of stand, although none of the differences between treatments, either for stand or tonnage of beets, was significant.

The width-of-row and spacing study (table 7) was conducted at Fort Collins in 1931. The seed was planted in hills with hand planters, the spacing being accurate. Competitive beets were harvested and tonnage yields were calculated on the basis of a perfect stand.

[5] "Block" as used by Fisher (*1*) is synonymous with "replicate" as used here.

In most cases the beets were lifted with a mechanical lifter. Samples for analyses were washed, and each beet was rasped with a Keil rasp for a sample of pulp. The cold-water digestion method was used for percentage of sucrose in the beet. For apparent coefficient of purity determination one-half of each beet was ground by a multiple-saw grinder and a sample of juice obtained from the pulp in a hydraulic press. The percentage of sucrose in the juice was then determined by Horne's dry lead method and the apparent coefficient of purity calculated as the ratio of sucrose percentage to the corrected Brix.

The regression of weight of beets on stand was determined from the following formula:

$$W=\overline{w}+b(s-\overline{s}),$$

where W is the estimated weight of beets for any particular stand; $\overline{w}$ and $\overline{s}$, the means for weight and stand, respectively; s, any observed stand; and b, the regression coefficient, calculated from

$$\frac{\text{Covariance } ws}{\text{Variance } s} \text{ or } \frac{S(ws-\overline{n}\overline{s})}{S(w-)\overline{w}^2},$$

where S represents summation, for all experiments except A–1, A–4, and E–1, in which, with large numbers of plots, the data were distributed into frequency surfaces, the regression function b being calculated from

$$\frac{S(s-\overline{s})(w-\overline{w})}{S(w-\overline{w})^2}$$

with no removal of block effects.

Statistical evaluations of the significance of differences were obtained in all cases. The standard errors for the 1930 studies were calculated for each group by means of the formula

$$S.E.=\sqrt{\frac{(fd)^2}{n-1}}$$

where d=deviation from the mean, f=frequency, and n=number of individuals. Generalized standard errors were calculated for the competition studies (tables 4 and 5), and for the spacing study (table 7) by means of Fisher's "analysis of variance" (*1*). Unless otherwise stated, the 5-percent point was used as a minimum level of significance, the odds being at least 19 to 1 before significance is established.

The standard errors for mean weight per root for the 1930 results are given for each determination in tables 2 and 3. Since sucrose determinations were made only on bulked lots, standard-error calculations were not possible. The standard errors with the z test and difference necessary for significance for the 1931 results are given in tables 4 and 5.

EXPERIMENTAL RESULTS

ANALYSIS OF COMPETITION STUDIES

The results obtained are presented in tables 1 to 5. Table 1, which shows a general summary of results, includes only the number of beets taken and their weights in percentages of the check. For convenience in discussion, the four locations given in table 1 are designated A, B, C, and D.

TABLE 1.—*Summary of results obtained from competition studies conducted at four locations in Colorado, 1930 and 1931*

Type of competition	1930						1931					
	Barlow (A)			College (B)			College (C)			Rocky Ford (D)		
	Number of beets	Weight (percentage of check)	Sucrose (percentage of check)	Number of beets	Weight (percentage of check)	Sucrose (percentage of check)	Number of beets [1]	Weight (percentage of check)	Sucrose (percentage of check)	Number of beets [1]	Weight (percentage of check)	Sucrose (percentage of check)
1 corner blank	450	103.0	99.2	35	107.9	98.7	28	103.3	100.7			
2 corner blanks	61	107.8	100.8	33	118.4	104.0	18	109.4	97.9	18	123.5	97.7
4 corner blanks							22	109.5	95.1	17	121.3	91.4
1 blank in adjacent row	223	113.0	99.2	80	111.5	101.3	18	105.4	99.3	18	130.9	97.1
1 blank in same row	203	126.8	99.2	97	120.8	102.0	31	138.6	95.8			
2 blanks in adjacent rows	11	133.8	100.8				24	111.9	96.5	18	120.3	96.6
2 blanks in same row	14	162.4	101.5	41	141.3	99.4	20	162.0	95.8	19	150.7	95.4
Blanks on 3 sides				45	203.4	92.3	23	181.6	92.4	22	178.7	94.8
Blanks on 4 sides	[2] 7	201.9	96.2	33	180.2	97.4	25	202.8	93.1	21	194.9	97.7
Doubles [3] surrounded by singles				88	107.3		22	105.1	97.9	18	129.6	100.0
Singles between doubles, same row							17	79.5	101.4	15	94.7	98.9
Average singles and doubles alternated							39	92.3	100 0	33	112.3	99.4
Doubles alternated with triples [4]							17	85.5	97.2	14	109.9	100.6
Triples surrounded by doubles							16	75.1	98.6	16	124.8	102.9
Average doubles and triples alternated							33	80.3	97.9	30	117.3	101.7
Check (singles alone)	1,189	100.0	100.0				[5] 25	100.0	100.0	36	100.0	100.0

[1] Average number per plot, 5 plots at College farm, 6 plots at Rocky Ford. Doubles and triples were counted as number of hills.
[2] Includes plants adjacent to 3 blanks on Barlow farm.
[3] 2 beets per hill.
[4] 3 beets per hill.
[5] A different check with an average of 24 beets per plot was used for the comparison of doubles and triples alternated.

TABLE 2.—*Summary of results obtained on the Barlow farm (location A), Fort Collins, Colo., 1930*

Type of competition for beets harvested	Average number of beets	Weight of root		Percentage of sucrose	
		Grams	Percentage of check	Average percent	Percentage of check
1 corner blank	450	688± 16	103.0	12.9	99.2
2 corner blanks	61	720± 45	107.8	13.1	100.8
1 blank in adjacent row	233	755± 22	113.0	12.9	99.2
1 blank in adjacent row and 1 corner blank	90	841± 41	125.9	12.4	95.4
1 blank in same row	203	847± 24	126.8	12.9	99.2
1 blank in same row and 1 corner blank	75	800± 44	119.8	12.9	99.2
Between blanks in adjacent row	11	894±162	133.8	13.1	100.8
Between blanks in same row	14	1,085±108	162.4	13.2	101.5
1 blank in adjacent and one in same row	52	882± 42	132.0	13.1	100.8
1 blank in adjacent, 1 in same row, and 1 corner blank	17	861± 77	128.9	12.5	96.2
Adjacent to 3 or more blanks	7	1,349±159	201.9	12.5	96.2
Check (all beets with competition on 4 sides)	1,189	668± 10	100.0	13.0	100.0

TABLE 3.—*Summary of results obtained at College farm (location B), Fort Collins, Colo., 1930*

Type of competition for beets harvested [1]	Number of pairs	Root weight per beet			Percentage of sucrose		
		Test	Check	Percentage of check	Test	Check	Percentage of check
		Grams	*Grams*				
1 corner blank	35	572± 43	530±50	107.9	15.6	15.8	98.7
2 corner blanks	33	662± 50	559±46	118.4	15.5	14.9	104.0
1 blank in adjacent row	80	590± 30	529±32	111.5	15.4	15.2	101.3
1 blank in same row	97	691± 33	572±27	120.8	15.2	14.9	102.0
1 blank in same row (16 by 22 inches)	122	750± 30	595±25	126.1	14.3	14.9	96.0
Between blanks in same row	41	791± 62	560±48	141.3	15.3	15.4	99.4
Between blanks in same row (16 by 18 inches)	9	657±117	515±91	127.6	15.4	15.4	100.0
Between blanks in same row (16 by 20 inches)	44	625± 94	516±78	121.1	14.2	14.9	95.3
Between blanks in same row (16 by 22 inches)	50	834± 54	676±47	123.4	14.5	14.5	100.0
1 blank in same and 1 in adjacent row	72	812± 35	549±34	147.9	15.1	15.6	96.8
Blanks on 3 sides, 2 in same, 1 in adjacent row	45	948± 60	466±30	203.4	14.4	15.6	92.3
Blanks on 3 sides, 1 in same, 2 in adjacent rows	21	1,186± 70	644±70	184.2	13.9	14.5	95.9
Blanks on 4 sides	33	838± 73	485±41	172.8	14.7	15.1	97.4
Doubles surrounded by singles	88	736± 36	686±32	107.3	.....	.....	.....
Triples surrounded by singles	10	757± 71	560±59	135.2	.....	.....	.....

[1] Spacing and width of row 12 by 20 inches, unless otherwise specified.

TABLE 4.—*Summary of results obtained at College farm (location C), Fort Collins, Colo., 1931*

[Values are given as means of 5 plots and percentage of check]

Type of competition for beets harvested	Average number of beets per plot	Weight of tops per beet		Weight of roots per beet		Percentage of sucrose		Apparent coefficient of purity	
		Grams	Percentage of check	Grams	Percentage of check	Average	Percentage of check	Average	Percentage of check
1 corner blank	28	530	104.7	826	103.3	14.5	100.7	90.2	100.7
2 corner blanks	18	520	102.8	875	109.4	14.1	97.9	89.6	100.0
4 corner blanks	22	592	117.0	876	109.5	13.7	95.1	88.0	98.2
1 blank in adjacent row	18	538	106.3	843	105.4	14.3	99.3	90.5	101.0
1 blank in same row	31	647	127.9	1,109	138.6	13.8	95.8	88.0	98.2
Between 2 blanks in adjacent rows	24	566	111.9	895	111.9	13.9	96.5	89.1	99.4
Between 2 blanks in same row	20	715	141.3	1,296	162.0	13.8	95.8	88.0	98.2
Blanks on 3 sides, 2 in same, 1 in adjacent row	23	877	173.3	1,453	181.6	13.3	92.4	88.2	98.4
Blanks on 4 sides	25	990	195.7	1,622	202.8	13.4	93.1	87.2	97.3
Doubles surrounded by singles	[1] 22	565	111.7	841	105.1	14.1	97.9	89.3	99.7
Singles between doubles, same row	17	423	83.6	636	79.5	14.6	101.4	89.5	99.9
Average singles and doubles alternated	39	494	97.6	738	92.3	14.4	100.0	89.4	99.8
Check, singles alone (A)[2]	25	506	100.0	800	100.0	14.4	100.0	89.6	100.0
Doubles alternated with triples	[1] 17	508	112.4	625	85.5	13.9	97.2	89.8	100.6
Triples alternated with doubles	[3] 16	537	118.8	549	75.1	14.1	98.6	89.8	100.6
Average doubles and triples alternated	33	523	115.7	587	80.3	14.0	97.9	89.8	100.6
Check, singles alone (B)[4]	24	452	100.0	731	100.0	14.3	100.0	89.3	100.0
z		1.4087		2.4099		0.4722		0.3208	
5-percent point		.4066		.4066		.4066		.4066	
1-percent point		.5553		.5553		.5553			
Difference for significance		88		116		.73			
Standard error (percent of mean)		4.73		3.80		1.85		.82	

[1] Pairs.
[2] Applies to all treatments listed above in the table.
[3] Hills.
[4] Applies to the treatments included between checks A and B in the table.

TABLE 5.—*Summary of results obtained at Rocky Ford, Colo. (location D), 1931*

[Values are given as means of 6 plots and percentage of check]

Type of competition for beets harvested	Average number of beets per plot	Weight of roots per beet		Percentage of sucrose		Apparent coefficient of purity	
		Grams	Percentage of check	Average	Percentage of check	Average	Percentage of check
2 corner blanks	18	463	123.5	17.0	97.7	86.4	98.3
4 corner blanks	17	455	121.3	15.9	91.4	86.1	98.0
1 blank in adjacent row	18	491	130.9	16.9	97.1	87.2	99.2
Between 2 blanks in adjacent rows	18	451	120.3	16.8	96.6	86.8	98.8
Between 2 blanks in same row	19	565	150.7	16.6	95.4	87.7	99.8
Blanks on 3 sides, 2 in same, 1 in adjacent row	22	670	178.7	16.5	94.8	86.6	98.5
Blanks on 4 sides	21	731	194.9	17.0	97.7	87.5	99.5
Doubles surrounded by singles	18	486	129.6	17.4	100.0	87.8	99.9
Singles between doubles, same row	15	355	94.7	17.2	98.9	88.2	100.3
Average singles and doubles alternated	33	421	112.3	17.3	99.4	88.0	100.1
Doubles alternated with triples	14	412	109.9	17.5	100.6	87.5	99.5
Triples surrounded by doubles	16	468	124.8	17.9	102.9	87.2	99.2
Average doubles and triples alternated	30	440	117.3	17.7	101.7	87.4	99.4
Check (singles alone)	36	375	100.0	17.4	100.0	87.9	100.0
z		1.4926		−0.4640		0.2534	
5-percent point		.5265				.5265	
1-percent point		.7443					
Difference for significance		97					
Standard error (percent of mean)		7.36		1.99		.58	

It is possible, by applying the data presented in table 1, to determine the extent to which the beets adjacent to a single blank utilized the additional space. Since the essential data are not complete for location D, the results obtained for locations A, B, and C only are averaged. As an average of these three locations, a beet adjacent to 1 corner blank was increased 4.7 percent in weight, and a beet adjacent to 1 blank in the adjacent rows and 1 blank in the same row was increased in weight 10.0 and 28.7 percent, respectively. The eight beets surrounding a blank space were, therefore, so increased in weight that they compensated for 96.2 percent of the loss due to a single missing beet. It is obvious that several blank spaces together will result in proportionately less efficient utilization of the vacant area by the adjacent plants.

As an average of the A, B, and C locations, increases in weight of beet root of 28.7, 55.2, and 95.0 percent, respectively, were obtained for beets adjacent to 1 blank in the same row, between 2 blanks in the same row, and with blanks on 4 sides. The respective increases in area are 41, 82, and 100 percent. Corresponding slight decreases were obtained for percentage of sucrose, the average in percentage of the check being 99.0, 98.9, and 95.6 for the three groups. A beet adjacent to 2 blanks on the corner, 2 blanks in the adjacent rows, and 2 blanks in the same row showed increases in weight of 13.6, 22.0, and 58.4 percent, respectively, for an average of the A, C, and D locations. Corresponding sucrose values in percentage of the check, or 98.8, 98.0, and 97.6, were obtained. It is evident that while the weight of the beet was increased greatly there was only a slight negative response for percentage of sucrose and a possible similar response in apparent coefficient of purity with increasing space for development. The effect on the apparent coefficient of purity appears to be less than the effect on percentage of sucrose.

The effect of doubles was studied for the B, C, and D locations. In 1930, at the B location, doubles surrounded by singles were found to yield an increase in weight of marketable [6] beets of 7.3 percent over single beets surrounded by singles, which will be termed the "check" beets. Corresponding increases of 5.1 and 29.6 percent were obtained for the C and D locations. The C location at Fort Collins and the D location at Rocky Ford were planned so that singles and doubles were alternated in the row, while adjacent rows were thinned entirely to singles. The singles alone, when alternated in the same row with the doubles, yielded only 79.5 and 94.7 percent of the yields of check beets for these respective locations. When the average for doubles and singles was considered, the corresponding yields in percentage of the check were 92.3 and 112.3, or an average of 102.3 for the two results. Neither of these deviations from the yield of the checks is as great as twice the standard error of a difference; consequently, they cannot be considered significant. Under the conditions of this test, the tonnage yield of marketable beets was not significantly affected when 25 percent of all hills were doubles.

When doubles were alternated with triples in the row, with the adjacent rows consisting of doubles, there was a significant loss in weight per hill of marketable beets at Fort Collins for both the doubles and triples as compared with the check beets. In relation

[6] A beet 1½ inches or more in diameter is considered marketable. By test it has been found that beets less than 1½ inches in diameter have more than a 50-percent chance of being returned to the grower as "tare".

to the check, the corresponding weights of doubles and triples were 85.5 and 75.1 percent, respectively. At Rocky Ford, the respective weights for the doubles and triples were 109.9 and 124.8 percent of the checks. The increase in weight of triples over the check closely approached significance.

As a result of late planting and relatively unsatisfactory growing conditions at Rocky Ford the yield was unusually low as contrasted with somewhat better than average conditions at Fort Collins. The differences obtained for the two stations in relation to doubles and triples might well be expected under these conditions.

REGRESSION OF WEIGHT ON STAND

The regression of weight on stand was determined by means of data from the three sources, as discussed under materials and methods. The correlation and regression values are given in table 6.

On a perfect-stand basis, the respective yields on the three fields, A, E, and F, were 18.3, 20.4, and 19.2 tons per acre. They may be considered highly productive fields.

The correlation coefficients varied considerably, but were significant in every case as determined by Fisher's *t* test, with the 1-percent point as the minimum level of significance. It is apparent that there is a very material correlation between total weight of roots from a given area and the relative stand of beets. This should be of real concern to a commercial grower of sugar beets.

TABLE 6.—*Correlation and regression values for the relation between weight of beets and percentage of stand, Fort Collins, Colo., 1930 and 1931*

Year, experiment, and treatment [1]	Plots		*r*		Regression of weight on stand [2]		Mean percentage stand	Range in percentage stand
	Number	Size	Within blocks	Total	Within blocks	Total		
1930:								
A–1	1,600	1 row × 16 feet		+0.5389		1.31	85.3	25–113
A–2	800	1 row × 32 feet	+0.5533	+.5485	1.39	1.36	85.3	47–103
A–3	200	4 rows × 32 feet	+.3775	+.3526	.80	.76	85.3	69–98
A–4	1,600	1 row × 16 feet		+.6900		1.69	85.3	25–113
A–5	200	4 rows × 32 feet	+.5361	+.5824	1.24	1.33	85.3	69–98
1931:								
E–1	1,280	1 row × 16 feet		+.4386		1.08	89.0	31–100
E–2	160	4 rows × 32 feet	+.3454	+.1675	.76	.45	89.0	75–99
F [3]	75	8 rows × 60 feet	+.7098	+.7375	2.10	1.37	59.5	33–78

[1] Experiments A and E were uniformity trials and F was a fertilizer study. Trials A–1, A–2, and A–3 were made before correction, and A–4 and A–5 after correction for competition. (See section on materials and methods.)

[2] The regression value is given as tons of beets for each 10-percent variation in stand.

[3] The 4 central rows only were harvested for the F trial; in all other cases, the entire plot was harvested.

The regression of weight on stand has been converted into expected tons of beets for each respective 10-percent increase or decrease in stand. The total regression only was calculated for the original plots, 1 row 16 feet in length, for experiments A–1, A–4, and E–1. When two or more of the original plots were grouped into larger sizes of plots, then a certain arbitrary number of hypothetical treatments were assumed as follows: 20 treatments for A–2, and 5 treatments for A–3, A–5, and E–2, with 40 replicates in each case. There were 15 treat-

ments in the fertilizer trial, F, with 5 replicates of each treatment. Where treatments were assumed for the uniformity trials, A and E, or actually provided as in the fertilizer trial, it was then possible to assign certain portions of the variance to replicates and accordingly to reduce the total variance within replicates. The regression within blocks represents more nearly the true relationship since it is freed from the variation between blocks or replicates. In such cases, the regression was determined for both the total and within-blocks relationships. The regression within blocks was materially greater than the total regression for the E–2 and F experiments, indicating for these experiments considerable variation between blocks. There was apparently no appreciable block effect for the other studies.

The regression value is reduced when the plot size is materially increased for the uniformity trials, A and E, with a consequent increase of the range in percentage of stand. When the plot size for the A experiment was increased from 1 row 16 feet in length to 1 row 32 feet in length, the total regression value remained practically constant, being 1.31 and 1.36 tons, respectively, for each 10 percent in stand for the two plot sizes. An eightfold increase in size, however, for A–3 as compared to A–1, resulted in a decrease from 1.31 to 0.76 tons for each 10 percent in stand. Corresponding reductions in regression values from 1.69 to 1.33 tons were obtained for A–4 and A–5, and from 1.08 to 0.45 tons for E–1 and E–2, both of these constituting eightfold increases in size of plot.

It seems apparent on the basis of these results that each unit of increase or decrease in stand will result in a greater variation in yield when the stand is low than when a perfect stand is approached. Additional support for this conclusion is found in the graph of the straight regression lines (unbroken) for experiments A–1, E–1, and F (fig. 2). These lines were drawn for the percentage-stand arrays included in the test. By Fisher's t test, the deviations of the expected from the obtained means in each array were not significant for any of the three tests. The z values for the three tests (0.2003, 0.1770, and 0.2948) closely approached the expected 5-percent points for significance (0.2867, 0.2926, and 0.3520). With larger populations, particularly in the extreme arrays, it seems probable that significant deviations from a straight line could be shown.

It is evident (fig. 2) that the regression lines must pass through the zero points for percentage of stand and for tons of beets per acre. It is also evident that the yield of beets will not continue to increase indefinitely in direct proportion to the increase in percentage of stand. In fact, it appears for the width-of-row and spacing data, which are also presented in figure 2, that up to 8-inch spacing there is generally some increase in tonnage yields, with an indication of a slight reduction in yield from 8- to 6-inch spacing. The reduction in yield for 6-inch as compared to 8-inch spacing was not statistically significant in any case. It was definitely shown at Fort Collins (table 1), however, that when doubles and triples alternated in the row, with the border rows consisting of doubles, there was a significant loss in tonnage, and it may be concluded that the stand was too dense in this case for maximum tonnage. From these results, it would seem probable that with sufficient data covering the entire range of arrays i. e., from zero to 200- or 300-percent stand, it should be possible to show a gradual curve, with the weight of beets increasing directly

in proportion to the number of beets for the lower percentage stands. This rate of increase would become gradually less as competition became more of a factor until the maximum was obtained; then an actual decrease would result as the stand became more dense.

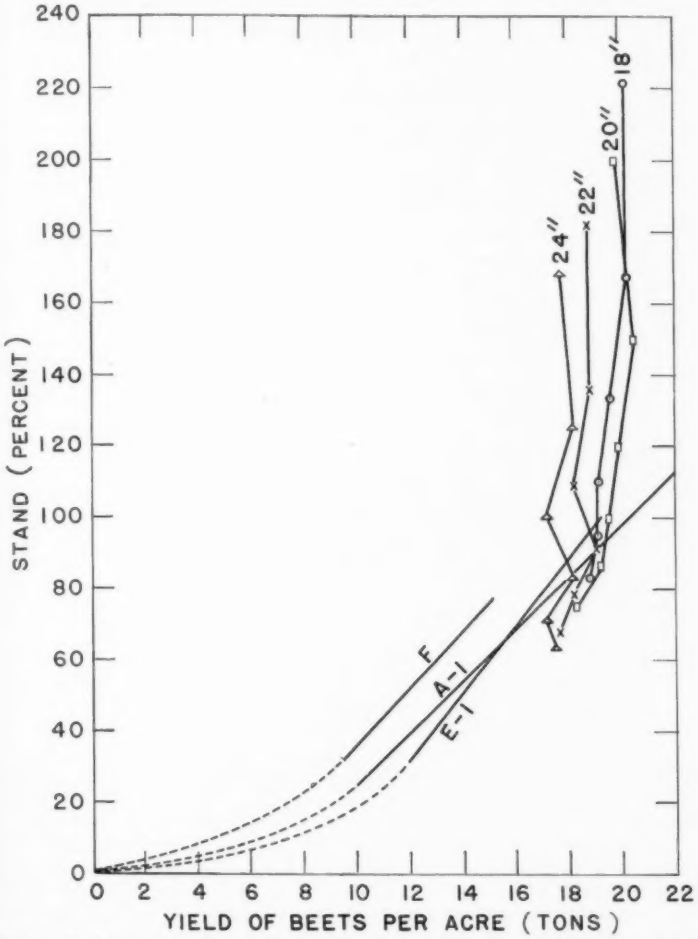

FIGURE 2.—Straight regression lines obtained for experiments A, E, and F (see table 6) with anticipated curvilinear projection (broken line) which intercepts the base lines for percentage of stand (26,136 beets per acre=100-percent stand) and tons of beets per acre at the zero point. The mean yields for the width-of-row and spacing study, which are given in table 7 and plotted similarly in this figure, and the different values for each width of row are connected with straight lines. The yield is to a much greater extent dependent upon the density of the stand when the variation in stand is due largely to the presence of missing plants or skips (A–1, E–1, and F) than when the variation is due to uniform differences in spacing (18-, 20-, 22-, and 24-inch widths of row).

By applying the Mitscherlich effect law to the data of Souček (*6*) and De Haan and Klijnhout (*2*) Willcox (*7*) has shown that a gradual curve is to be expected up to a certain point of density of

stand, but that further increases in stand are not expected to alter the yield.

When correction was applied, on the basis of known competition, to the individual plot yields for the 1930 uniformity trial (A, table 6), the linearity of relationship was somewhat improved (fig. 3), as shown by the sums of squares for deviations of the expected from the obtained mean-array values. The obtained z values for deviations from a straight line are 0.2003 and -0.0401 for before and after correction, respectively, with a 5-percent point of 0.2867 for each. As shown by figure 3, the obtained mean much more closely approached the expected when correction had been made. While it is quite evident that the correction instituted materially improved the linearity of relationship between stand and yield, it is not feasible to consider such an arduous and costly method for practical plot work.

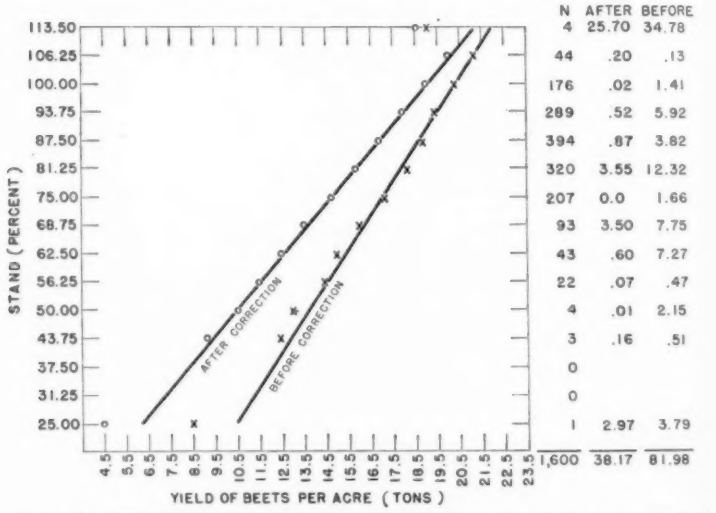

FIGURE 3.—Calculated straight regression lines for a uniformity trial at Fort Collins, Colo., 1930, with obtained mean yields in tons of beets per acre for each percentage-stand array. N, Number of observations; two right-hand columns show the sums of squares for deviations of the obtained from the expected for each percentage-stand array.

Results obtained in connection with a fertilizer experiment conducted on a farmer's field substantiate forcibly the relationship between the stand and tonnage yield. Strictly competitive beets, which were harvested from check or untreated plots in this particular experiment, averaged 13.54 tons per acre. The stand averaged 51 percent, and the field as a whole produced an average of 7.29 tons per acre, or 6.25 tons less than were obtained for the competitives. Assuming a regression of 1.25 tons of beets for each 10 percent in stand, the assumption being a fair one in relation to the results herein presented, a loss in tonnage of 6.13 tons per acre can be accounted for as due to a loss of 49 percent in stand. Figured at the local factory price paid to farmers for the 1932 crop, the farmer suffered a net loss of $29.56 per acre from this loss in stand. The initial stand was excellent, the loss being almost wholly due to lack of diligence and care in

blocking and thinning and later cultivation. With careful work, the farmer should have harvested almost a perfect stand and nearly doubled the tonnage which he obtained, thereby realizing a net profit for his capital and labor invested.

RELATIONSHIP BETWEEN YIELD AND STAND

The mean yields for each of the treatments used in the spacing study are presented in table 7.

TABLE 7.—*Yield of beets per acre for competitive beets in spacing study, Fort Collins, Colo., 1931*

[Values are given as means of 6 plots]

Width of row (inches)	Yield when the spacing was—						Average yield
	6 inches	8 inches	10 inches	12 inches	14 inches	16 inches	
	Tons	*Tons*	*Tons*	*Tons*	*Tons*	*Tons*	*Tons*
18	20.10	20.28	19.60	19.13	19.02	18.77	19.48
20	19.86	20.50	19.96	19.58	19.17	18.31	19.56
22	18.73	18.79	18.17	19.07	18.25	17.67	18.45
24	17.71	18.26	17.21	18.17	17.13	17.51	17.67
Average	19.10	19.46	18.73	18.99	18.39	18.06	

The obtained z values for spacing, width of row, and interaction of spacing with width of row were 0.4769, 1.1082, and −0.5156, respectively. The corresponding 5-percent points were 0.4176, 0.5950, and 0.4176, indicating significance for only spacing and width-of-row differences. It is obvious that the 18- and 20-inch widths of row produced a heavier tonnage of beets than the 22- and 24-inch widths when the stand was approximately the same. It is also apparent that, in general, the larger yields were obtained from the 8-inch spacing. The tonnage for the 6-inch spacing dropped off slightly, although not significantly, for each width of row. The increase in yield from the 8-inch spacing over that from the 12- and 16-inch spacing is shown in table 8.

There were 50 percent more beets per acre for the 8- than for the 12-inch spacing, and 100 percent more for the 8- than for the 16-inch. The corresponding average yield increases for the four widths of row were 0.47 and 1.39 tons of beets per acre, with a difference of 0.85 tons per acre necessary for significance. The 0.47-ton difference between the 8- and 12-inch spacing does not approach significance, while the 1.39-ton increase of the 8-inch over the 16-inch is highly significant. This provides a striking contrast to the regression within blocks, which ranges from 0.76 to 2.10 tons of beets per acre for each 10-percent increase in stand (table 6), the essential difference being that the variation in stand in the spacing study is due to uniform space allotment and also very largely to the presence of skips in the regression studies. The obvious conclusion is that uniformity of stand is a relatively far more important factor in determining final yield than is the particular width of row or spacing used. While it is not essential to this phase of the study, it is of interest to record that, although the tonnage of beets per acre is larger for the narrower spacings, the individual beets obtained were smaller and slightly higher in percentage of sucrose and purity.

TABLE 8.—*Yield of beets per acre from 8-inch spacing as compared with yield from 12- and from 16-inch spacing, Fort Collins, Colo., 1931*

Width of row (inches)	Increase or decrease (—) of yield per acre from 8-inch spacing as compared with that from—	
	12-inch spacing	16-inch spacing
	Tons	*Tons*
18	1.15	1.51
20	.92	2.19
22	—.28	1.12
24	.09	.75
Average	.47	1.39

DISCUSSION

The results reported indicate that the sugar beet is capable of marked response to variations in available space for development. Weight of beet is influenced to a much greater extent by an increase in the space allotment per plant than is either percentage of sucrose or apparent coefficient of purity.

In agronomic experiments with sugar beets the stand may be influenced by the type of treatment or differential response between varieties. Experiments which seek to determine the value of such factors as frequency and depth of cultivation, machine blocking as compared to hand blocking, time of planting, fertilizers or fertilizer-placement methods, irrigation, spacing studies, and thinning practices may be expected to show differences in stand between treatments with resulting variations in weight and quality of individual beets. In such cases the stand must be held constant in order to obtain a measure of the effect of the treatment. This may be obtained by harvesting only those beets which are surrounded by a full stand, i. e., normally competitive beets.

In actual practice it is clearly advantageous to have the beets either mechanically blocked or spaced by hand with reference to a marked tape. Metal boxing tape marked with lacquer is very useful for this purpose. It has been customary in the agronomic experiments to insist on competition on four sides. The data (table 1) indicate that although there is small response from 1 corner blank, 2 or more corner blanks may result in as large an error as 1 adjacent blank in the adjacent row. It is essential, therefore, to consider the presence or absence of beets on the corners in critical studies.

From the grower's standpoint it is of considerable interest to be able to present the yields on the acre basis regardless of the nature of the stand. This information may be obtained in addition to the perfect-stand data by harvesting the normally competitive beets (which are surrounded by perfect stand) separately from the noncompetitive beets (which lack competition in the same or adjacent rows). In practice the noncompetitive beets may be topped sufficiently with a hoe just previous to harvest to mark them. When lifted the competitive beets are first removed from the plot, leaving only the noncompetitive beets to be taken up as a separate lot. The samples for

laboratory determinations are usually taken from the competitive beets in order to assure comparable results.

The regression of weight of beets on stand, if linear, would be useful in adjusting yields to a uniform-stand basis for treatment comparisons. From the regression values presented, it appears that this relationship was essentially linear within the observed stand limits. It is apparent, however, that this relationship would have been nonlinear with wider variations in stand (fig. 2).

The practical importance of skips in the stand was shown in the regression of weight of beets on stand. Three separate studies were made to demonstrate this relationship. Each 10-percent increase in the number of beets per unit area resulted in a corresponding increase of 0.76 to 2.10 tons of beets per acre (regression within blocks, table 6). This is in striking contrast with the results obtained from a spacing study (table 8), where there was an increase of 0.47 ton of beets per acre for a 50-percent increase in stand (8- over 12-inch) and 1.39 tons for a 100-percent increase (8- over 16-inch) when the spacing was consistent and uniform. These results demonstrate clearly the necessity of uniform stands for the commercial grower of sugar beets. The placement of major emphasis on uniformity of stand involves almost every field practice, particularly those operations preceding and immediately following blocking and thinning and, to a somewhat lesser extent, cultivation, irrigation, and protection against insect pests.

SUMMARY AND CONCLUSIONS

The studies here reported were conducted near Fort Collins, Colo., in 1930 and 1931, and at Rocky Ford, Colo., in 1931. The object was to determine the response of sugar beets to increased space allotment per plant, and the relationship which exists between weight and density and uniformity of stand.

On the basis of 2 years' results obtained at three locations, it was determined that the beets surrounding a single blank space were increased in weight to compensate for 96.2 percent of the loss of a single beet.

As an average of the three locations, increases in root weight of 28.7, 55.2, and 95.0 percent were obtained for beets adjacent to 1 blank space in the same row, between 2 blank spaces in the same row, and with blank spaces on 4 sides, respectively. The respective sucrose values in percentage of the check were 99, 98.9, and 95.6 percent, and increases in area 41, 82, and 100 percent.

It is evident from the data presented that beet weight was greatly influenced by the relative area available for development. As the size of the beet increased in response to increased spacing allotment, there was a slight negative response in percentage of sucrose and possibly in the apparent coefficient of purity, although the effect on the apparent coefficient of purity appears to be less than that shown for percentage of sucrose.

When doubles were alternated with singles in the same row and the adjacent rows consisted entirely of singles, the weight of the singles adjacent to doubles in the row appeared to be reduced slightly. The respective yields for the two locations of singles alternated with doubles were 79.5 and 94.7 percent of the check. The corresponding

percentage yields of 92.3 and 112.3 when both doubles and singles were included did not differ significantly from the check yield for singles.

At Fort Collins a significant decrease in tonnage resulted from alternating doubles and triples, the border rows consisting of doubles. The respective yields for the doubles and triples were 85.5 and 75.1 percent of the singles. At Rocky Ford the total yield for doubles and triples was greater than the check, although not significantly so.

Weight of beets was found to be significantly correlated with percentage of stand, the r values ranging from $+0.3454$ to $+0.7098$ for the relationship within blocks.

The regression of weight of beets on stand proved to be essentially linear for the three experiments, although in each case the deviations from regression were such that the obtained z values were only slightly less than the expected values for the 5-percent point. It is probable from the data presented that, with a wider range of stand, nonlinearity could be demonstrated. For each increase of 10 percent in stand, there was a corresponding increase ranging from 0.76 to 2.10 tons of beets per acre for regression within blocks.

Yields obtained in connection with a study of width of row and spacing showed increases of an average of 0.47 ton for the 8- over the 12-inch spacing, or an increase of 50 percent in number of beets per acre, and 1.39 tons for 8- over 16-inch, or a 100-percent increase in number of beets per acre, the spacing being accurate and uniform. Uniformity of space allotment, or, in other words, the elimination of skips or blank spaces in the stand, appears to be relatively a far more important factor in determining final yields than the particular width between rows or the spacing between beets in the row.

Practical suggestions are made for harvesting experimental plots of sugar beets in order to eliminate the effect of variations in stand.

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STUDIES ON PROPERTIES OF THE CURLY TOP VIRUS [1]

By C. W. BENNETT [2]

Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The determination of properties of the curly top virus is somewhat more difficult than are similar determinations with many other viruses, as the percentage infection from artificial inoculation is too low to afford a critical test for the presence or absence of virus. The unsatisfactory results from artificial inoculations have forced investigators to rely on the natural insect vector, *Eutettix tenellus* (Baker), for the production of any considerable amount of infection.

Experimental work on the properties of the curly top virus was facilitated by the development of a method by Carter (*5*) [3] for artificially feeding the beet leaf hopper. This method consists in placing a liquid containing the virus in a bag made of animal membrane and allowing nonviruliferous leaf hoppers access to the outside of the bag. The leaf hoppers puncture this membrane and feed sufficiently on the liquid to acquire the virus. In this way the beet leaf hopper may be utilized to transfer virus from the liquid medium to susceptible tissue of beet plants.

Modifications of Carter's original method of feeding the beet leaf hopper have been devised and used with considerable success in studies on the properties of the virus by those interested in this field of research. Severin and Swezy (*14*) determined that the virus is filterable, and recently Severin and Freitag (*13*) published results of further investigations on properties of the virus. In the latter work it was shown that the virus may be recovered from expressed beet juice after a period of aging ranging from 8 to 100 days, the period apparently depending on the previous treatment of the juice and on the conditions under which the virus was preserved. Virus was recovered from beet juice diluted 1:2,000 and from macerated leaf hoppers diluted 1:22,000. The virus was inactivated by a 10-minute exposure at 80° C., but temperatures considerably below freezing apparently had no injurious effect. No virus was recovered from dried plant tissue nor from dried beet leaf hoppers.

Studies on the properties of the curly top virus were started at Riverside, Calif., the latter part of 1932 and were continued during the following 2 years. In this paper certain methods which have greatly facilitated the experimental work are described and the results of the investigation are presented.

METHOD OF MAKING TESTS FOR VIRUS

In all of the tests for the presence of active virus in this series of studies, beet leaf hoppers were used as the agents of transfer of virus from the liquid medium to seedling beets. Several types of cages for

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[2] The writer is indebted to J. M. Fife, biochemist, Division of Sugar Plant Investigations, Bureau of Plant Industry, for making the hydrogen-ion determinations reported in this paper.

[3] Reference is made by number (italic) to Literature Cited, p. 240.

feeding leaf hoppers were tried, one of which, designed for feeding the insects with small quantities of exudate obtained from the beet plant, proved very satisfactory for use with all kinds of virus-containing media. Cages of this type were used for the most part and were constructed as follows:

Each feeding cage was made from an ordinary 4-ounce bottle having a diameter of approximately 2 inches. The bottle was cut into three parts and the top part discarded. The two remaining parts were ground smooth on the cut surfaces. The middle portion, covered at one end with cloth and at the other end with Baudruche capping skin, served as a cage for confining the leaf hoppers. The bottom portion was used as a moist chamber over which the cage was set. Nonviruliferous beet leaf hoppers were placed in the cage through an opening in the cloth cover and subjected to a starvation period of 2 to 18 hours, depending on the temperature. At the expiration of the starvation period the cage was inverted and drops of the material to be tested for virus content were placed on the capping skin. The cage was then quickly righted, so as not to disturb the drops of medium, and placed over a moist chamber (fig. 1). The cages were placed under lights in a hood at a temperature of 95° to 110° F. and the leaf hoppers allowed a feeding period of 4 to 6 hours. The leaf hoppers were then removed and, except as noted, caged singly on seedling beets. The number of plants infected under these conditions was taken as a relative measure of the virus content of the material on which the leaf hoppers had fed.

FIGURE 1.—Cage used in artificial feeding of the beet leaf hopper.

SOURCES OF VIRUS

BEET JUICE

Juice expressed from sugar beet plants affected with curly top has been used as the source of virus in nearly all of the studies on properties reported up to the present time. This beet juice, when fed directly to the beet leaf hopper, is not an especially favorable liquid from which to obtain virus, since only a small percentage of nonviruliferous beet leaf hoppers become viruliferous as a result of such feeding. Some estimate of the proportion of leaf hoppers that may be expected to acquire virus from beet juice may be obtained from

results selected from one of the experiments of Severin and Freitag (*13*), which seems to be representative. Using approximately 33 artificially fed leaf hoppers for inoculating each plant, they obtained 4.6 percent infection from leaf juice, 46.6 percent from root juice, 11.5 percent from leaf juice diluted with 5-percent sugar solution, and 4.3 and 42.1 percent, respectively, from leaf and root juice centrifugalized at high speed. These results indicate that even with the higher percentages of infection the actual number of the artificially fed leaf hoppers which picked up virus was small, since it is well known that a single viruliferous leaf hopper is able to transmit disease to a considerable percentage of the plants on which it feeds.

The limited capability of the leaf hopper for picking up virus from beet juice is further illustrated by the results shown in table 1, in which less than 4 percent of the total number of leaf hoppers given access to leaf and root juice and caged singly on seedling beets transmitted disease.

TABLE 1.—*Relative availability to beet leaf hoppers of virus in leaf juice, root juice, and phloem exudate of sugar beets affected with curly top*

Beet no.	Leaf juice		Root juice		Phloem exudate	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
1	80	3	80	5	80	26
2	20	0	----	----	20	5
3	20	0	20	0	20	7
4	20	0	20	1	20	14
5	20	1	20	0	20	14
Total	160	4	140	6	160	66

Direct utilization of the expressed juice of diseased plants, because of the limited feeding which takes place, the high mortality of the insects, and other circumstances, involves the use of excessively large numbers of beet leaf hoppers to secure results, and the results are often contradictory and difficult of interpretation. Nevertheless, the method may be used successfully in the study of certain properties of the curly top virus. It is obviously unsuited for studies in which chemical agents on which the leaf hopper will not feed or which are injurious to the leaf hopper are added to the liquid containing the virus. In any type of property studies the method involves the exercise of a great amount of patience and effort and necessitates the use of enormous numbers of leaf hoppers.

Because of these obvious shortcomings of beet juice as a source from which beet leaf hoppers may acquire virus, the first efforts in this series of property studies were directed toward securing liquids of high virus content more acceptable to the beet leaf hopper than beet juice has proved to be. Attempts were made (1) to obtain the liquid content of the phloem of the beet and other plants in which there is reason to believe virus occurs in relatively high concentrations and (2) to separate the virus in expressed beet juice from materials that are toxic or unacceptable to the beet leaf hopper.

PHLOEM EXUDATE

According to the available evidence (*2*) the curly top virus occurs in the phloem of diseased plants in much greater concentrations than in other tissues. Since it has been shown also that the beet leaf hopper seeks the phloem elements in its feeding, the phloem content of diseased plants should constitute the best possible medium from which to recover virus when the beet leaf hopper is used as the agent of transmission.

Many varieties of cucurbits are susceptible to the curly top disease. Since these plants are known to give off considerable quantities of exudate from the phloem when the stems or petioles are cut, preliminary tests of phloem exudate as a virus-carrying medium were made with Hubbard squash (*Cucurbita maxima* Duchesne) as the source of exudate. However, it was found that the exudate from this and other types of cucurbits coagulates quickly upon exposure to air and even when considerably diluted with water or 5-percent sugar solution it is not a favorable food for the beet leaf hopper. After having access to the phloem exudate from squash for 4 hours the leaf hoppers were weak and emaciated. However, of the 120 leaf hoppers exposed for 4 hours to this material and then caged singly on healthy seedling beets, 15 produced infection.

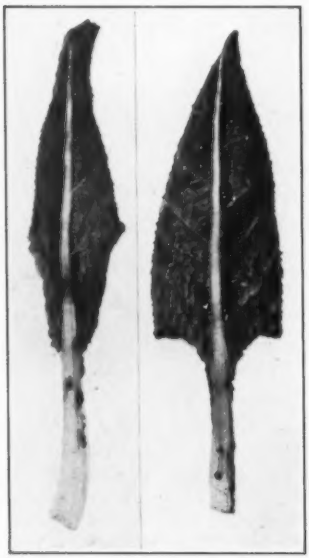

FIGURE 2.—Exudate on diseased beet leaves.

Attempts were made next to obtain exudate from the phloem tissue of sugar beets, *Beta vulgaris* L. Under certain conditions of rapid growth and high humidity, drops of clear or colored liquid appear on the blades and petioles of diseased beets (fig. 2). A small quantity of this exudate was collected by means of capillary tubes and tested by allowing leaf hoppers to feed on it and then transferring them singly to seedling beets. This exudate proved to have a high virus content and, if diluted sufficiently with water, served as a very satisfactory food for the beet leaf hopper. Exudate can be obtained only in very limited quantities from diseased blades and petioles. However, it was found that by properly regulating environmental conditions exudate could be obtained in larger quantities from the cut surface of both diseased and healthy beet roots.

After considerable testing of conditions which favor exudation the following method was adopted as best suited to this purpose. Beets 2 to 6 inches in diameter were removed from the soil, washed, topped, placed in sand in a container, and covered to avoid excessive evaporation. The tops of the beets projected 2 to 3 inches above the surface of the sand. The sand was kept wet but sufficient drainage was pro-

vided to prevent water-logging. A thin layer of tissue was removed from the top of each beet. On the cut surface drops of exudate appeared above the veins as shown in figure 3. These drops were collected by means of small glass tubes. Beets, if freshly cut, will yield exudate each day for as long as 3 weeks. The largest yield was always obtained after the first cut, and the volume of flow decreased progressively as other cuts were made. As a rule it is not desirable to cut beets more than once or twice each day. There is no evidence that the virus content of the exudate is influenced by the number of times the beet is cut or by the time it stays in sand so long as it remains sound. Tests showed that the leaf hoppers fed readily on this exudate and that a large percentage acquired virus, as was proved by their ability to cause infection. Table 1 shows comparative results in acquiring virus by lots of leaf hoppers allowed to feed on exudate from sugar beets affected with curly top and on expressed juice from leaves and roots of the beets from which the exudate was derived.

FIGURE 3.—Drops of exudate on cut surface of diseased beet.

The available evidence as to the origin of the exudate obtained as described above indicates that it is derived chiefly from the phloem. Crafts (7) and others have shown that a positive pressure normally exists in the phloem of certain plants and that liquid content escapes when phloem is tapped. The appearance of drops of exudate on the petioles and blades of diseased beets suggests that phloem content is extruded to the exterior as a result of pressure. Esau (8) presented results of histological studies which show that liquid moves to the surface

from the region of phloem through the intercellular spaces, indicating strongly that the natural exudate on blades and petioles is derived from the phloem.

The exudate from the cut surface of beets collects in beadlike drops at the severed ends of the vascular bundles, indicating clearly a vascular origin. Evidence has been presented (*2*) indicating the nonoccurrence of virus in the xylem elements of the vascular bundle. The high virus content of the exudate from sugar beets affected with curly top, therefore, is evidence that it is not derived from the water-conducting tissue but from the phloem. Moreover, exudation is not of the type which commonly results from injured xylem. Exudation from the cut surface of beets continues for a period of 30 minutes to 2 hours following cutting. Further exudation can be induced only by making fresh cuts. This suggests a closing of the outlets by coagulable materials of the phloem, a phenomenon that occurs to a more marked degree in cucurbits where, after injury, exudation continues for only a few minutes but can be induced repeatedly by making fresh cuts. Bleeding from the xylem would be expected to continue over a longer period.

The exudate from the cut surface of diseased beets closely resembles the natural exudate from the petioles in viscosity, hydrogen-ion concentration, oxidation reactions as indicated by color changes, total solids, and virus content. It seems logical to assume that naturally occurring exudate is derived from the phloem and that the exudate from the cut surface of beets is made up of the content of phloem tissues except for a certain amount of unavoidable contamination from injured cells of other tissues mainly parenchymatous in nature. The high virus content of phloem exudate of curly top diseased beets and its acceptability to the beet leaf hopper make this material an extremely satisfactory source of curly top virus. It has been used extensively in all experiments in which only small quantities of virus-containing liquid were required. In experiments requiring a considerable volume of liquid other materials have been used.

PRECIPITATES FROM BEET JUICE

Separation and purification studies by a number of investigators have shown that viruses have an affinity for certain types of precipitates formed upon the addition of certain chemical compounds to plant juices. Allard (*1*) found that the virus of tobacco mosaic is contained in the precipitate formed from tobacco juice when acetone or alcohol is added. Vinson (*16*) developed a method for purifying the virus of tobacco mosaic from the precipitate formed when safranine is added to the expressed juice of tobacco.

The addition of alcohol or acetone to beet juice results in the production of a precipitate that is easily removed by centrifugalization. It was found that this precipitate contained considerable virus that was released in a supernatant liquid after the precipitate was dried to remove the alcohol, suspended in water, and centrifugalized. Making use of this principle, the following method was developed for preparing virus-containing liquids for leaf hopper feeding. Juice from diseased beets is added to an equal volume of 95-percent alcohol. The resulting precipitate is thrown down by centrifugalization and the supernatant liquid, which contains most of the materials of the juice that

are unacceptable to the beet leaf hopper, is discarded. The precipitate is washed in 50-percent alcohol, dried, suspended in 5-percent sugar solution, and centrifugalized. The supernatant liquid from this second centrifugalization contains considerable virus and is very acceptable to the beet leaf hopper. The percentage of leaf hoppers that acquired virus from such a medium varied with the different preparations but was sufficiently high to justify the use of the medium in experiments in which phloem exudate could not be used because of the relatively large quantities of liquid required.

It was found that this method of separating the virus from materials that interfere with leaf hopper feeding could be used also with phloem exudate, ground beet leaf hoppers suspended in water, and with other virus-containing liquids which have proteins or other compounds that are precipitated in an alcoholic solution. The method has been of considerable value in separating the virus from toxic materials of the beet juice and from liquids to which various chemical compounds were added to determine their effect on the virus.

PRECIPITATION AND PURIFICATION

A number of tests were made to determine the completeness of the removal of the virus by the precipitate formed in beet juice and phloem exudate upon the addition of alcohol or acetone, and also to determine insofar as possible the completeness of release of the virus when the precipitate is thoroughly shaken in water or in 5-percent sugar solution. For these tests juice was pressed from badly diseased beet tops that previously had been frozen. A volume of 95-percent alcohol equal to the volume of juice was added and the resulting precipitate thrown down by centrifugalization. The precipitate was washed once with a volume of 50-percent alcohol equal to twice the volume of juice. It was then dried and made up to the original volume of juice with 5-percent sugar solution. This was shaken well and centrifugalized, and the supernatant liquid was reserved for testing. The precipitate was washed with 5-percent sugar solution a second and a third time. Tests were made to compare, as a virus source, (1) the original beet juice, (2) supernatant liquid after alcoholic precipitation, (3) supernatant liquid from the 50-percent alcoholic wash, (4) supernatant liquid from the first 5-percent sugar solution wash, (5) supernatant liquid from the second 5-percent sugar solution wash, and (6) supernatant liquid from the third 5-percent sugar solution wash. The alcohol was removed by evaporation from the supernatant liquid and the precipitate in (2) and (3).

Table 2 shows the results of a series of tests in which nonviruliferous leaf hoppers were fed on these preparations. As in previous experiments, the leaf hoppers were able to obtain very little virus from the untreated beet juice. No virus was obtained from the supernatant liquid following the alcoholic precipitation and little was recovered by washing the precipitate with 50-percent alcohol. These two liquids were evaporated to dryness and the residues made up to the original volume of beet juice with 5-percent sugar solution. They were not particularly distasteful to the leaf hoppers, although they were not so acceptable, apparently, as the other preparation. The fact that under these conditions the leaf hoppers picked up very little virus indicates that the removal of virus from the leaf juice by alco-

holic precipitation is practically complete and also that the precipitate may be washed in 50-percent alcohol without appreciable loss of virus.

A considerable quantity of virus is released from the alcoholic precipitate when it is washed with a 5-percent sugar solution, but a considerable quantity also is retained as shown in the tests of the second and third 5-percent sugar solution washes. Tests in which the second and third washes were used as virus sources gave almost as much infection as was obtained by using the first wash.

Experiments similar to the foregoing were made with phloem exudate as the original source of virus. As in the foregoing experiment, the removal of the virus by precipitation with alcohol was almost complete. The precipitate was washed six times with 5-percent sugar solution. Tests indicated that there was almost as much virus in the sixth wash as in the first.

Less extensive experiments indicate that acetone is about as effective as alcohol in precipitating the virus. Virus is released from the acetone precipitate, as from the alcoholic precipitate, upon the addition of water or 5-percent sugar solution.

TABLE 2.—*Leaf juice compared with alcoholic precipitate of leaf juice subjected to different treatments, as a source of virus for beet leaf hoppers*

[20 plants were used in each test with each type of inoculum]

Test no.	Plants infected from indicated virus source [1]				
	Leaf juice	Alcoholic wash of precipitate [2]	First wash of precipitate [3]	Second wash of precipitate [3]	Third wash of precipitate [3]
	Number	*Number*	*Number*	*Number*	*Number*
1	0	0	5	1	0
2	0	0	9	5	1
3	0	0	11	17	10
4	0	1	13	11	11
5	0	1	11	7	3
6	1	0	11	16	14

[1] No infections resulted when supernatant liquid from alcoholic precipitate was used as source of virus.
[2] Alcohol was removed by evaporation.
[3] 5-percent sucrose solution was used for washing.

When alcoholic precipitates of beet juice or phloem exudate are suspended in 5-percent sugar solution and centrifugalized, the supernatant liquids usually retain enough material in suspension to produce a slight cloudiness. Some material remained in suspension even after prolonged centrifugalization. However, visible suspensoids were removed by passage of the supernatant liquids through Berkefeld and Mandler candles, and since the filtrates contained active virus, these visible suspensoids are apparently not essentially associated with the virus, although they may have some correlation with virus concentration in these preparations.

Virus-separation tests were made also by the safranine precipitation method developed by Vinson (*16*) for purifying tobacco mosaic virus. Juice expressed from previously frozen leaves of diseased beets was centrifugalized to remove the coarser particles. Five cubic centimeters of a 1-percent aqueous solution of safranine was added to 25 cc of expressed juice. The resulting precipitate was thrown down in a centrifuge, washed with a volume of water equal to that of the juice, and

suspended in 25 cc of distilled water. One gram of Lloyd's alkaloidal reagent was added, and the mixture was shaken at intervals for 30 minutes. It was then centrifugalized and the supernatant liquid, if almost clear, was poured off and tested for virus content. If safranine was still present in too great a quantity, the treatment with Lloyd's reagent was repeated. In most instances it was possible to obtain a straw-colored or slightly pink liquid. One gram of sugar was added to 25 cc of supernatant liquid, and tests were made for virus content by means of nonviruliferous leaf hoppers. Other tests were made in which phloem exudate was used instead of beet leaf juice.

TABLE 3.—*Virus available to beet leaf hoppers in supernatant liquids from different precipitates*

Source of supernatant liquid for virus tests	Plants inoculated	Plants infected
	Number	*Number*
Safranine precipitate	60	1
Alcohol precipitate	60	28
Acetone precipitate	60	20
Alcohol precipitate and Lloyd's reagent	20	14

The results (table 3) indicate that the leaf hoppers were able to acquire very little virus from the supernatant liquid from the safranine precipitate. As controls on the technic, supernatant liquids from alcohol and acetone precipitates were used and it was found that the leaf hoppers acquired considerable virus. The reason for the failure to obtain more virus from the supernatant liquid of the safranine precipitate is not clear. The leaf hoppers fed reasonably well on this material, though not so satisfactorily as on the supernatant liquids from the alcohol and acetone precipitates. This poorer feeding on the part of the leaf hoppers was apparently due to something released from Lloyd's reagent, since 1 g of Lloyd's reagent shaken in 25 cc of supernatant liquid prepared from an alcohol precipitate showed lower infections than an alcohol precipitate treated similarly except for the use of Lloyd's reagent. However, from this latter liquid leaf hoppers acquired considerable virus and it seems unlikely that failure to acquire more virus from the supernatant liquid following the safranine precipitate was due entirely to a lesser volume of liquid that may have been taken up by the leaf hoppers. It seems probable either that the virus is not carried down in the safranine precipitate or that it is not released from the precipitate upon the addition of Lloyd's reagent. Because of the difficulties inherent in the use of leaf hoppers as agents in transmission, no tests adequate to solve this problem have been devised.

Other tests show that the precipitates formed as a result of the addition of such reagents as calcium chloride and hydrochloric acid and the precipitate formed by heating juice or exudate to 60° C. carry down the virus. As judged from the percentage of infection, however, the amount of virus obtained from such precipitates has been apparently much less than from precipitates formed by the addition of alcohol or acetone.

DILUTION TESTS

A study of the infectivity of different dilutions of extracts of diseased plants and of ground viruliferous beet leaf hoppers was undertaken primarily to determine the dilutions of virus-carrying media that could best be used in further experimental work. In view of the fact that phloem exudate, the material that had proved to be the best source of virus, is available only in limited quantities, it was especially important to know how much this material could be diluted without seriously decreasing virus availability to leaf hoppers. The following materials and procedures were used in preparing liquids of different virus dilutions for leaf hopper feeding.

(1) Phloem exudate was collected from the cut surface of badly diseased beets and dilutions were made with 5-percent sugar solution.

(2) Beet juice was expressed from badly diseased beets selected from greenhouse material. A known volume of juice was added to an equal volume of 95-percent alcohol. The resulting precipitate was thrown down by centrifugalization, washed once with 50-percent alcohol, dried, and made up to the original volume of juice with 5-percent sugar solution. The precipitate was suspended in the sugar solution and dilutions were made direct from this mixture.

(3) One-tenth of a gram of viruliferous beet leaf hoppers was ground in a mortar and mixed with 2 cc of distilled water. The mixture was centrifugalized and the supernatant liquid was added to 2 cc of 95-percent alcohol. The resulting precipitate was thrown down by centrifugalization, washed once with 50-percent alcohol, dried, and suspended in 1 cc of 5-percent sugar solution. This was considered a 1:10 dilution and was used as the basis for higher dilutions.

Nonviruliferous leaf hoppers were allowed to feed on different dilutions of these preparations of phloem exudate, beet juice, and beet leaf hoppers, and, except in the case of high dilution, as noted in table 4, they were caged singly on seedling beets. The following numbers of plants were inoculated from each dilution, using one leaf hopper per plant: 184 from phloem exudate, 160 from beet juice, and 120 from beet leaf hoppers. The results of the dilution tests with phloem exudate are shown in table 4. The infection percentages obtained from dilutions of phloem exudate, beet juice, and leaf hoppers are shown in figure 4.

TABLE 4.—*Effect of dilution of phloem exudate on the number of artificially fed leaf hoppers that acquired and transmitted virus*

Magnitude of dilution	Plants infected by—		Magnitude of dilution	Plants infected by—		Magnitude of dilution	Plants infected by—	
	1 leaf hopper per plant [1]	10 leaf hoppers per plant [2]		1 leaf hopper per plant [1]	10 leaf hoppers per plant [2]		1 leaf hopper per plant [1]	10 leaf hoppers per plant [2]
	Number	*Number*		*Number*	*Number*		*Number*	*Number*
None	57		1:50	53		1:10,000	0	4
1:5	72		1:100	37		1:20,000		1
1:10	76		1:500	18		1:50,000		0
1:20	60		1:1,000	9	13			

[1] 184 plants were inoculated in each test.
[2] 40 plants were inoculated in each test.

Assuming that the percentage infection from different dilutions of phloem exudate, beet juice, and beet leaf hoppers, shown in figure 4, roughly represents comparative virus concentration, it is evident that the virus content of phloem exudate is much greater than that of beet juice and somewhat greater than that of the beet leaf hopper.

The maximum dilutions of beet juice from which virus was obtained correspond closely to those from which Severin and Freitag (*13*) consistently obtained virus, though the maximum dilutions from which they occasionally obtained virus are somewhat higher than the maximum reported here. These investigators showed that the maximum dilutions of beet juice from which virus was obtained by feeding the leaf hoppers directly on the beet juice varied with the method of preparation and treatment of the juice before leaf hopper feeding. In three types of juice treatment the maximum dilution from which they obtained virus was 1:100 from untreated juice as expressed

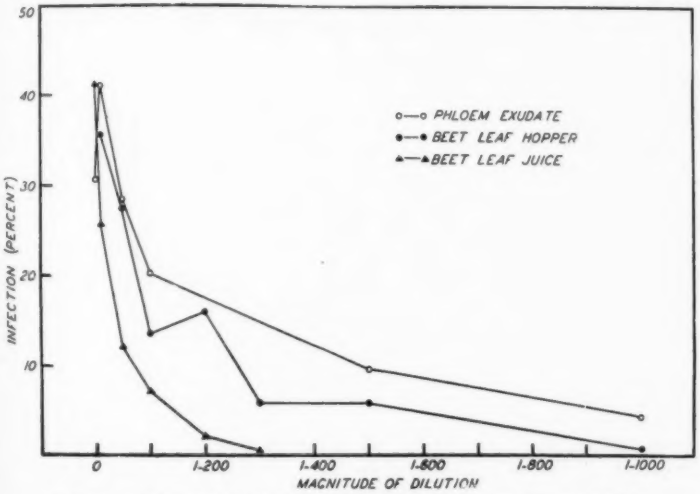

FIGURE 4.—Influence of dilution of virus from different sources on curly top infection.

from the plant, 1:1,000 from centrifugalized juice, and 1:300 from juice passed through a Berkefeld V or W filter. In each case a slight dilution was more favorable for virus recovery than was undiluted juice.

In comparing the results obtained in these tests it should be noted that Severin and Freitag (*13*) made their inoculations by caging approximately 33 artificially fed leaf hoppers on each plant. In the writer's tests only one insect was caged on each plant. The number of insects used to inoculate the test plant apparently influences infection and determines to some extent the maximum dilution from which virus may be recovered. This is illustrated by the results in table 4, which show that with phloem exudate as the virus source and by using 1 leaf hopper for inoculating each plant, virus was obtained from a dilution of 1:1,000 but not from a dilution of 1:10,000. By using 10 leaf hoppers for inoculating each plant, virus was obtained from dilutions of 1:10,000 and 1:20,000 but not from a dilution of 1:50,000.

These results indicate that mass action may be a factor influencing infection. Evidence of the operation of this phenomenon in infection by the curly top virus was first presented by Carsner and Lackey (*4*), who postulated a minimum infective dosage of virus. If it were assumed that some or all of the leaf hoppers that feed on a 1:10,000 dilution of phloem exudate acquired a small amount of virus but that the amount acquired in each instance was below the minimum required for infection, it follows that no infection would result from leaf hoppers caged singly, assuming no increase of virus in the insect. However, if 10 of these leaf hoppers were caged on a single plant the sum of the virus aliquots introduced by the different leaf hoppers might reach the minimum dosage necessary for infection. Although the results presented here are much too meager to justify conclusions, the method of approach seems to hold possibilities for an attack on some of the phases of the mass-action problem.

FILTRATION TESTS

Severin and Swezy (*14*) demonstrated that the virus in the juice of beets affected with curly top and that present in an aqueous suspension from macerated viruliferous beet leaf hoppers readily pass the common types of "bacteria-proof" filters. Since this work proves the filterable character of the curly top virus, it has not been thought worth while to make further extensive filtration tests. Such experiments as are recorded here were made preliminary to tests of longevity of the virus in filtered and unfiltered media.

For this preliminary work phloem exudate was diluted 1 part exudate to 9 parts 5-percent sugar solution and passed through Berkefeld N and W and Mandler medium and fine filters, under a pressure equivalent to 10 cm of mercury. The filters had previously been sterilized, and the filtrate was collected in sterile flasks. The filtrates were placed in small sterile test tubes in quantities of approximately 3 cc per tube. About 1 cc of liquid was removed aseptically from each tube for testing for virus content. The tubes with the remaining liquid were set aside to serve as checks on the presence of bacterial contaminants. Since phloem exudate is an excellent medium for bacteria, it is considered that the presence or absence of bacterial growth in these tubes furnished an accurate index as to whether the filters used were effective in holding back bacteria and other microorganisms.

TABLE 5.—*Filterability of curly top virus through various types of filters*

[20 plants were inoculated from each filtrate in each test]

Test no.	Plants infected by virus filtered as indicated				
	Check [1]	Berkefeld N	Berkefeld W	Mandler medium	Mandler fine
	Number	*Number*	*Number*	*Number*	*Number*
1	3	7	7	8	6
2	7	8	4	3	4
3	13	8	7	10	7
4	3	1	3	4	3

[1] Unfiltered.

The results of four series of tests (table 5) show that the virus in phloem exudate passed all the grades of filters used. The comparative numbers of infections do not indicate that any amount of virus measurable by this method is held by the filters. The virus apparently passes the finer grades of filters to about the same degree as it passes the coarser ones.

In further tests it was found that the virus in leaf juice, root juice, water wash of alcoholic precipitate of leaf juice, and water wash of alcoholic precipitate of phloem exudate readily passed the ordinary filters such as the Berkefeld N or Mandler medium grade.

RESISTANCE OF VIRUS TO AGING IN LIQUID MEDIUM

Numerous tests have shown that the virus in expressed beet juice retains its activity for 1 to 7 days at ordinary temperatures, but, in some instances, it may remain active for as long as 14 days. The factors responsible for these variations and those influencing inactivation are very obscure. However, since the curly top virus occurs sparsely, if at all, in parenchymatous tissue, it may well be that parenchyma cells contain materials inimical to prolonged activity of the virus. Bacterial action and products of oxidation in expressed juice are also factors which must be considered in this connection.

An experiment was planned in an attempt to separate and, if possible, evaluate these factors suspected of being important in inactivation. Badly diseased leaves from young beets were frozen and the juice expressed and centrifugalized to remove the coarser particles. The juice was then divided into four lots and treated as follows: Lot 1, tubed; lot 2, passed through a sterile Berkefeld N filter, and aseptically placed in sterile tubes; lot 3, water wash of alcoholic precipitate prepared and tubed; lot 4, water wash of alcoholic precipitate prepared, passed through a sterile Berkefeld N filter, and placed aseptically in sterile tubes.

The four lots of material were incubated at room temperature and the tubes were removed from each lot at intervals for testing the virus content. For each test a water wash of alcoholic precipitate of the contents of the tubes in lots 1 and 2 was prepared. A small amount of sugar was added to this and also to the tubes of lots 3 and 4 before the feeding of leaf hoppers. After a feeding period of approximately 4 hours the leaf hoppers were caged singly on seedling beets.

Three series of tests were made. In these there was considerable variation in infection and some variation in the time the virus remained active in the three series, but the conclusions which may be drawn from any one of these tests are in accord with those which may be drawn from the combined results of the three tests shown in table 6. These results show that in the unfiltered leaf juice the virus remained active for 7 days but not for 14 days. In the filtered leaf juice in which the virus was not subjected to the action of micro-organisms, the virus remained active for the same length of time as in the unfiltered juice. In this case micro-organisms apparently had no effect on the virus. It will be noted that the infection from filtered juice was much less than from any of the other three lots. Considerable difficulty was experienced in obtaining sufficient filtrate for tests because of the formation of a covering of colloidal material on the outside of the filter. This material probably interfered with passage of the virus and may account for the smaller number of infections.

In the unfiltered water wash of the alcoholic precipitate of leaf juice the virus was active after 14 days but not after 21 days. In the filtered wash of the alcoholic precipitate the virus was active after 28 days but not after 35 days. In the unfiltered water washings of the alcoholic precipitate micro-organisms apparently had an influence in virus inactivation. The increased tolerance to aging in both the filtered and unfiltered wash of the alcoholic precipitates as compared to the leaf juice suggests that the elimination of the materials present in leaf juice but not precipitated by alcohol considerably increases the time the virus will remain active in a liquid medium. In the leaf juice these materials may inactivate the virus before the full influence of bacterial action would be exerted and this may account for the fact that the virus remained active as long in unfiltered as in filtered juice.

TABLE 6.—*Resistance of virus to aging in filtered and unfiltered leaf juice and in filtered and unfiltered wash of alcoholic precipitate of leaf juice*

[60 plants were inoculated in each test]

Virus source	Plants infected after indicated number of days							
	0	1	3	7	14	21	28	35
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Leaf juice not filtered	33	31	28	4	0	0	0	0
Leaf juice filtered	13	14	12	1	0	0	0	0
Wash of precipitate not filtered	39	25	25	8	4	0	0	0
Wash of precipitate filtered	22	23	34	23	8	9	3	0

INFLUENCE OF DESICCATION ON LONGEVITY OF VIRUS

In the tests to determine the resistance of the curly top virus to desiccation several types of materials containing virus were selected. These were dried and kept at room temperature for the period of the test. Liquids for testing for the presence of the virus in the dried material were prepared at intervals by mixing the dried materials with water or with 5-percent sugar solution or by making extracts of the dried material acceptable to the beet leaf hopper. Nonviruliferous beet leaf hoppers were allowed to feed on these liquids and caged singly on seedling beets. The infection that resulted was accepted as a measure of the virus content of the dried material. The following materials and methods were used.

Phloem exudate.—Exudate from the cut surface of diseased beet roots was diluted 1 part exudate to 4 parts distilled water, and 6 drops of the mixture was placed on each of several capping skins attached to the bottom of cages for feeding leaf hoppers. The drops were dried at room temperature before an electric fan. A liquid for making tests of virus content was prepared by adding a drop of distilled water to each bit of dry residue. In a second test naturally occurring phloem exudate, dried to a sirupy consistency, was scraped from diseased beet petioles and placed in an open container at room temperature. At intervals a liquid for testing virus content was prepared by adding a small amount of the dry residue to 1 cc of distilled water. In a third test the alcoholic precipitate from phloem exudate was dried at room temperature and kept in an open container. Test liquids were prepared by mixing a small amount of the dry residue with 1 cc of 5-percent sugar solution.

Sugar beet plants.—Severely diseased sugar beet plants having 6 to 8 true leaves were selected from greenhouse material. Considerable exudate was present on the petioles and blades. The plants were divided into two lots. The superficial exudate was removed from the first lot by washing but was allowed to remain on

the second lot. The plants were dried in a current of air at a temperature of about 100° F. In each test for virus content six plants were pulverized in a mortar and steeped in a quantity of cold distilled water equal to approximately twice the green weight of the plants. The water was expressed and added to an equal volume of 95-percent alcohol. The precipitate resulting was thrown down by centrifugalization, washed once with 50-percent alcohol, and dried. It was then made up to a volume equal to half the volume of water added to the dried plants, with 5-percent sugar solution. This was shaken and centrifugalized, and the supernatant liquid was used as the test liquid.

Alcoholic precipitate of beet juice.—Juice was expressed from the tops and roots of diseased beets. The juice from each source was divided into lots of 5 cc each and precipitated with alcohol. The precipitate was washed once with 50-percent alcohol and dried. The dried material was preserved and tests were made at intervals by grinding each lot of dried material in 5 cc of 5-percent sugar solution, centrifugalizing, and using the supernatant liquid for feeding leaf hoppers.

Beet leaf hoppers.—Viruliferous leaf hoppers were killed by etherization, dried at about 100° F., and preserved in a vial. At regular intervals 50 leaf hoppers were removed from the vial, ground in a mortar, and mixed with distilled water. The mixture was centrifugalized and the supernatant liquid was precipitated with alcohol. The precipitate was washed once with 50-percent alcohol, dried, made up to 1 cc with 5-percent sugar solution, and centrifugalized, and the supernatant liquid was used to feed leaf hoppers.

Tests of virus content of the preparations just described were made at monthly intervals. The results of these tests are shown in table 7. As might be expected in tests of this type, there was considerable variation in the percentage of infection from month to month. These fluctuations were increased by a resistant variety of beet accidentally used in some of the tests. In spite of these fluctuations, it is evident that the virus remained active for long periods in dry material and that the nature of the medium from which the virus was taken influenced longevity.

TABLE 7.—*Effect of desiccation on longevity of the curly top virus*

[20 plants were inoculated in each test]

Dried material as virus source	Plants infected by virus dried for indicated number of months										
	Check	1	2	3	4	5	6	7	8	9	10
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Exudate from cut surface of beet	15	7	16	9	9	13	4	0	0	2	2
Natural exudate	10	14	-----	11	5	3	3	1	7	7	10
Alcoholic precipitate of exudate	8	7	6	4	7	3	0	0	0	0	0
Beet tops (washed free of exudate)	13	9	5	3	1	0	0	0	0	0	0
Beet tops (not washed free of exudate)	16	9	6	2	2	0	0	0	0	0	0
Alcoholic precipitate of leaf juice	6	3	2	0	0	0	0	-----	-----	-----	-----
Alcoholic precipitate of root juice	9	2	1	0	0	0	0	-----	-----	-----	-----
Beet leaf hoppers	7	3	7	4	4	2	2	-----	-----	-----	-----

There was little measurable decrease in quantity of active virus in natural phloem exudate in a period of 10 months, and a certain amount of active virus was still present in exudate from the cut surface of diseased beets after 10 months. The virus remained active for much shorter periods in all materials in which parenchyma tissue or products of parenchyma tissue occurred. Since all of the material used in these experiments was kept in the open at room temperature, the actual moisture content varied with that of the surrounding air. It is probable that the moisture content was sufficient to permit the action of any deleterious substances contained in the samples. The period of activity of the virus in preparations

containing parenchyma or products of parenchyma may have been influenced by the action of substances deleterious to the virus and contained in the parenchyma. It is significant in this connection that natural exudate, the material in which the virus retained its greatest activity, had least contact with parenchyma, since it probably escaped directly from the phloem and passed to the surface through the intercellular spaces.

EFFECT OF TEMPERATURE

LOW TEMPERATURE

Tests of the time the curly top virus will withstand temperatures below freezing have been made with the virus contained in phloem exudate as it is derived from diseased beets and also with exudate diluted 1 part exudate to 4 parts 5-percent sugar solution. Small glass tubes were filled with these materials, sealed at both ends, and placed in a refrigerator held at approximately −6° C. The tubes were removed and their contents tested at monthly intervals, in the usual way, by means of nonviruliferous beet leaf hoppers. The results of these tests (table 8) indicate that the virus retains its activity for at least as long as 18 months.

TABLE 8.—*Effect of prolonged periods of freezing on virus activity*

[20 plants were inoculated in each test]

Virus-bearing material	Plants infected by virus frozen for indicated number of months											
	Check	1	2	3	4	6	7	8	9	11	12	18
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Phloem exudate not diluted	6	16	12	----	14	----	----	----	9	----	4	----
Phloem exudate diluted 1 : 4	8	15	13	13	14	16	12	8	14	2	6	3

During the course of other experiments it was repeatedly found that the virus retained its activity for prolonged periods in a variety of frozen materials contained in test tubes, 25-cc flasks, and open beakers. No difficulty has been experienced in obtaining active virus from materials such as exudate, water wash of alcoholic precipitate of exudate, or water wash of alcoholic precipitate of beet juice, after a storage period of several months. Advantage has been taken of this fact to collect relatively large quantities of virus-containing substances for subsequent experimental work. These stored materials have afforded a more uniform source of virus for repetition of experimental work and comparison of results than otherwise would have been available.

ALTERNATE FREEZING AND THAWING

Attempts were made to determine the effect of alternate freezing and thawing on the virus contained in phloem exudate and also on the virus contained in the water wash of the alcoholic precipitate of leaf juice. These materials were placed in small test tubes in 1-cc quantities. The tubes were placed in the freezing compartment of a refrigerator held at approximately −6° C. The tubes were removed

daily and the contents thawed at room temperature and returned to the refrigerator. Tubes which served as checks were kept frozen continuously until tested. Tests of virus content were made at weekly intervals. When the experiment was terminated at the end of 3 weeks, there was no measurable decrease in virus concentration in either type of material and apparently the quantity of active virus is not measurably diminished by such a treatment.

HIGH TEMPERATURES

In order to determine temperatures at which the virus would remain active, tests were made with phloem exudate diluted 1 part exudate to 4 parts 5-percent sugar solution. Thin-walled glass tubes with a maximum diameter of about 2 mm were filled with this diluted exudate and sealed at both ends. The tubes were subjected to the desired temperature ($\pm 0.5°$ C.) in a water bath for 10 minutes. After treatment, the tubes were removed and the contents were made available to nonviruliferous leaf hoppers which were later caged singly on seedling beets.

Table 9 shows the results of five series of tests. Under the conditions of these tests the thermal inactivation point of the curly top virus lies between 75° and 80° C. Perhaps the most interesting feature of these results is the rather small variation in temperature at which inactivation occurred in the different lots of exudate. In the four tests in which 1° temperature intervals were used, inactivation occurred at 80°, 79°, 76°, and 78°, respectively.

Tests similar to those just described were made in which the time of exposure to high temperatures was increased to 1 hour. In these tests leaf hoppers obtained virus from material subjected to 60°, 65°, and 70° C. but not from that subjected to 75° and 80°.

TABLE 9.—*Effect on the sugar beet curly top virus of 10 minutes' exposure to various temperatures*

[20 plants were inoculated from exudate treated at each temperature in each test]

Test no.	Plants infected by virus exposed for 10 minutes to indicated temperature (° C.)									
	Check	60	65	70	75	76	77	78	79	80
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1	7	9	11	8	7					
2	16				19	14	13	11	3	0
3	12				11	8	10	2	0	0
4	15				3	0	0	0	0	0
5	5				4	6	1	0	0	0

ATTENUATION OF VIRUS

In the thermal-inactivation-point tests just described it was observed that the plants infected by virus treated for 10 minutes at 76° to 79° C. grew well and produced relatively mild symptoms of disease. The leaves showed a medium amount of curling and the petioles were only slightly or not at all shortened. In contrast, the plants infected by the untreated virus were severely affected and remained small. The leaves were badly curled, the petioles were short, and several of the plants died before the experiments were terminated. Representative differences in growth are shown in figure 5.

On three series of plants inoculated with virus subjected to the differential temperatures, records of the incubation period of the disease, severity of symptoms, and dry weight of the plants after 6 to 8 weeks were made. These data for one experiment are shown in table 10. The results are similar to results obtained in the other two experiments except that in one experiment the heat treatments did not so markedly influence the period of incubation of the disease. It will be noted that the incubation period of the disease and the dry weight of affected plants tended to increase and the severity of symptoms tended to decrease as the temperature to which the virus was subjected was increased. These results show that the virus was attenuated to a considerable degree by the heat treatments and that attenuation occurs after a very short period of exposure.

In 2 of the 3 experiments virus was transferred to healthy plants from the checks and from representative plants affected by virus from

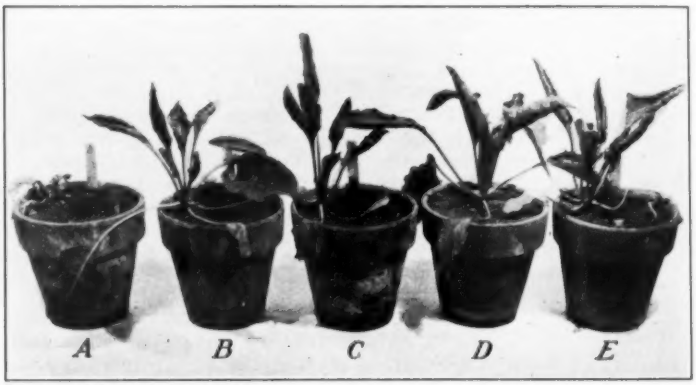

FIGURE 5.—Effect of 10-minute heat treatment on curly top symptoms produced in beet plants. The virus with which these plants were inoculated was treated for 10 minutes at the following temperatures: *A*, Untreated; *B*, 76° C.; *C*, 77°; *D*, 78°; *E*, 79°.

different heat treatments. In one test the passage of the virus was continued through four lots of healthy plants. Relative differences in severity of disease were maintained throughout these tests, indicating that the virus in its attenuated form was relatively stable.

TABLE 10.—*Attenuation of the curly-top virus by heat*

Temperature of 10-minute treatment (° C.)	Plants inoculated	Plants infected	Average incubation period	Average severity [1]	Average weight of diseased plants
	Number	*Number*	*Days*	*Grade*	*Grams*
(2)	19	16	10.2	4.4	0.26
75	20	19	11.3	3.3	.47
76	20	14	12.5	1.8	.71
77	20	13	13.1	1.2	.57
78	20	11	18.0	1.8	.79
79	20	3	19.3	1.6	1.13
80	20	0			

[1] Five grades of severity were used, plants in grade 1 being the least severely affected and those in grade 5 being the most severely affected.
[2] Virus untreated.

The rapidity with which attenuation was effected in the experiments just described is in striking contrast to results obtained in attenuation of other viruses at high temperatures. Johnson (*11*) reported attenuation of tobacco mosaic virus in tobacco plants exposed to a temperature of 35° to 37° C. for 10 days. Cucumber mosaic virus in tobacco plants was attenuated after a 10-day exposure to 37°. The question arises as to whether the curly top virus might be attenuated also at temperatures below 76° to 79° if the period of exposure were increased.

EFFECT OF HYDROGEN-ION CONCENTRATION

Tests were made to determine the effect of hydrogen-ion concentration on the virus contained in phloem exudate. Solutions buffered at approximately even pH units from 2.0 to 10.0, inclusive, were prepared according to the method of Clark (*6*). Measured quantities of phloem exudate were added to the buffered solutions in the proportion of 1 part exudate to 4 parts buffer. The mixtures were thoroughly shaken and allowed to stand for 2 hours. At the expiration of this time a portion of the liquid from each mixture was removed for a hydrogen-ion determination and the remainder was placed on a membrane to be fed on by nonviruliferous beet leaf hoppers. The hydrogen-ion determinations of the mixtures of the virus with buffers ranging in reaction from pH 2.0 to 7.0 were made by means of the quinhydrone electrode. Determinations on solutions of higher pH value were made by means of the glass electrode. The leaf hoppers were allowed a feeding period of 4 hours. Thus the virus was subjected to the reaction of the medium for a minimum period of 2 hours and a maximum period of 6 hours, the actual time depending on the time during the feeding period at which it was taken up by the leaf hoppers. At the end of the 4-hour feeding period the leaf hoppers exposed to the 2 liquids of greatest acidity and those exposed to the 2 liquids of greatest alkalinity were noticeably weaker than the individuals of other lots. The results of three series of tests are shown summarized in experiment 1 of table 11.

TABLE 11.—*Effect of a 2-hour treatment of virus in buffer solutions of different hydrogen-ion concentrations*

Experiment 1 [1]		Experiment 2 [2]	
Reaction of buffer solution (pH)	Plants infected	Reaction of buffer solution (pH)	Plants infected
	Number		*Number*
2.4	0	2.7	0
2.9	0	3.4	2
4.0	1	4.4	21
5.0	5	5.1	15
5.9	6	6.1	19
6.8	20	7.0	21
7.6	29	7.4	4
8.4	31	8.4	14
Check [3]	21	9.1	9
		Check	16

[1] 60 plants inoculated.
[2] 40 plants inoculated.

Additional tests made were similar to those of experiment 1 except that the exudate, after standing in contact with the buffer for 2 hours, was adjusted to a reaction of approximately pH 6.0 and precipitated with alcohol, and that a water suspension of the precipitates was prepared for the leaf hoppers. This provided for a definite exposure for 2 hours to the indicated pH and in each case supplied a uniform medium for the leaf hoppers, thus eliminating the unfavorable liquids of the more acid and the more alkaline treatments of the first tests. The results are shown in experiment 2 of table 11.

The results of these two experiments show a certain amount of unexplained variation in the number of plants infected at the different hydrogen-ion treatments. They agree, however, in that no virus was obtained from liquids in which the virus had been subjected to a reaction of pH 2.9 or lower. Infection from mixtures in which the exudate with the respective buffers was fed direct to the leaf hopper increased with the rise in alkalinity, despite the fact that the mixtures of higher pH values were obviously unfavorable as food for the leaf hopper. The reasons for the erratic results from the treatments at pH values of 7.4 and above in experiment 2 have not been determined definitely. As shown in the amount of infection at corresponding pH values in the tests of experiment 1, the virus is not inactivated at these reactions. It seems probable that the explanation may be found in an incomplete precipitation of the virus or in a close union of virus with the precipitate which prevented its being acquired by the leaf hoppers in quantities representative of its real concentration.

EFFECT OF CHEMICAL REAGENTS

ACETONE AND ALCOHOL

Tests were made with acetone and ethyl alcohol to determine the effect of different concentrations of these reagents on the virus. In these tests phloem exudate was made up to the desired concentration of reagent by the addition of acetone or absolute alcohol direct to the exudate. In the pure acetone and absolute alcohol treatments the exudate was dried before the reagent was added. The tubes containing the exudate in various concentrations of acetone and alcohol were corked, shaken well, and set aside for a period of 2 hours. At the expiration of this time the contents of the tubes were poured into watch glasses and evaporated to dryness. The residues were taken up in a volume of 5-percent sugar solution equal to five times the original volume of exudate. Nonviruliferous leaf hoppers were given access to this material for a period of 4 to 5 hours and then caged singly on seedling beets. The infection obtained was taken as a measure of the virus content of the medium on which the leaf hoppers fed.

The results of these tests (table 12) indicate that the curly top virus has a very great degree of tolerance to high concentrations of both acetone and alcohol. Of all the treatments used, only absolute alcohol seemed to have any deleterious effect on the virus. Even in absolute alcohol it was not completely inactivated by a 2-hour exposure.

TABLE 12.—*Effect of a 2-hour treatment with different concentrations of alcohol and acetone on the virus in phloem exudate*

[20 plants were inoculated at each concentration in each test]

Reagent and test no.	Plants infected by virus treated with reagent of indicated concentration (percent by volume)										
	Check	10	20	30	40	50	60	70	80	90	100
Alcohol:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1	13	13	17	16	16	13	15	16	16	15	2
2	8	12	16	13	15	14	17	17	13	17	2
3	8	7	9	9	5	5	13	13	11	8	4
Acetone:											
4	15	.....	16	.....	20	.....	13	.....	16	.....	14
5	9	.....	.....	.....	.....	.....	.....	.....	.....	.....	7

After it was found that the virus retained its activity after a 2-hour treatment with the various concentrations of acetone and alcohol used in the experiment just described, tests were made to determine how long the virus would remain active in different concentrations of these two reagents. Measured quantities of exudate were placed in small test tubes. Sufficient acetone or alcohol was added to make concentrations of 25, 50, and 75 percent of these reagents in the resulting mixtures. Tests of active virus content were made at intervals by taking measured quantities of material from the tubes holding the different concentrations, and evaporating to dryness. As in previous experiments, the residues were taken up in sugar solution and tests for virus activity were made with nonviruliferous leaf hoppers.

A second test was made with beet juice in 25-, 50-, and 75-percent alcohol. The mixtures were kept in stoppered flasks at room temperature. In making the tests for virus, the contents of the flasks were shaken well, after which enough liquid was poured out to correspond to 1 cc of the juice. This was centrifugalized and the supernatant liquid discarded. The precipitate was washed once with 50-percent alcohol, dried, mixed with 1 cc of 5-percent sugar solution, and centrifugalized, and the supernatant liquid was used for testing.

TABLE 13.—*Longevity of virus in phloem exudate and beet leaf juice in different concentrations of alcohol and acetone*

[20 plants were inoculated in each test]

Virus source and reagent	Concentration of reagent in mixture	Plants infected by virus treated for indicated number of days								
		0	1	3	7	14	21	28	35	56
Phloem exudate:	*Percent*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Alcohol	25	10	6	8	8	7	6	0	2	0
	50	6	9	8	10	7	1	5	3	3
	75	5	8	8	4	9	8	4	2	1
Acetone	25	12	11	9	5	5	3	0	1	0
	50	10	6	11	6	5	6	1	0	0
	75	9	9	5	7	0	3	0	1	2
Beet leaf juice:										
Alcohol	25	11	8	11	0	0	0	0	0	.....
	50	13	9	9	2	0	0	0	0	.....
	75	13	2	2	1	0	0	0	0	.....
Beet leaf juice	.....	8	6	10	0	0	0	0	0	.....

The results of these tests are shown in table 13. The longevity of the virus was apparently not influenced by the concentration of alcohol regardless of whether phloem exudate or beet juice was the original source of the virus. However, longevity of the virus was markedly influenced by the kind of plant material from which the virus was derived. Where phloem exudate served as the source of virus, active virus was present after 56 days in two of the alcohol preparations. Where beet juice was present, the virus was inactivated in 14 days, a period corresponding to the period of inactivation in beet juice without alcohol. This is further evidence indicating the presence of an inactivating substance in beet juice not present or present only in low concentrations in phloem exudate. Several tests have indicated that when alcohol or acetone is added to phloem exudate from beet the virus remains active 2 to 4 weeks longer than in similar material to which nothing is added. This indicates not only that acetone and alcohol fail to exert any appreciable deleterious effect on the virus but that they may function to protect the virus somewhat from the full effects of factors which, in the absence of acetone or alcohol, normally cause fairly rapid inactivation.

The resistance of virus contained in phloem exudate to the lethal action of alcohol is apparently rather marked as compared to other plant viruses. Fajardo (*9*) found that the virus of bean mosaic loses its activity in 50-percent alcohol in less than 5 minutes. Allard (*1*) showed that the tobacco mosaic virus is inactivated very quickly in concentrations of alcohol greater than 50 to 55 percent and that it remains active for less than 30 minutes in a concentration of 80-percent alcohol. Allard also found that with tobacco mosaic virus alcohol was more destructive than acetone.

GERMICIDES

Such common germicides as bichloride of mercury, copper sulphate, formaldehyde, and carbolic acid were used in a series of tests to determine their effects on the virus. Since the beet leaf hopper must be used to transfer the virus to the test plants, it was necessary after treatment to separate the virus from the reagents before passing it through the leaf hoppers. It was found that with all of these reagents except formaldehyde this separation could be made by removing the virus in the precipitate formed upon the addition of alcohol. Measured quantities of phloem exudate were placed in centrifuge tubes. An equal volume of reagent made up to twice the concentration desired in the final mixture was added. The mixture was thoroughly agitated and allowed to stand at room temperature for 2 hours. At the end of this time, except in tests where formaldehyde was used, 95-percent alcohol was added to each tube in amount equal to the volume of liquid already in the tube. The tubes were shaken and allowed to stand until a precipitate was formed. This usually required from 5 to 10 minutes. The precipitate was thrown down in a centrifuge and the supernatant liquid, containing most of the reagent, was discarded. The residue was washed once with 50-percent alcohol to remove additional quantities of reagent. It was then dried to remove the alcohol, after which it was made up to five times the original volume of exudate with 5-percent sugar solution, and the tests were made for virus content as in previous experiments. In the

formaldehyde tests the exudate, after a 2-hour treatment with formaldehyde, was evaporated to dryness at room temperature and then taken up in 5-percent sugar solution.

TABLE 14.—*Effect of a 2-hour treatment of virus in phloem exudate with different concentrations of germicides*

[20 plants were inoculated at each concentration in each test]

Reagent and test no.	Plants infected by virus treated with reagent of indicated concentration								
	1:25	1:50	1:100	1:200	1:300	1:400	1:500	1:1,000	Check
Copper sulphate:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1	.....	0	0	0	.....	.....	13	14	16
2	0	0	0	2	12	15	14	11	12
3	0	0	0	0	1	0	6	16	12
Bichloride of mercury:									
1	.....	.....	.....	.....	.....	.....	.....	13	15
2	.....	7	4	7	.....	.....	10	11	18
3	0	1	2	1	1	3	2	1	5
Formaldehyde:									
1	.....	.....	.....	.....	.....	.....	.....	11	13
2	.....	0	0	1	.....	.....	3	6	5
3	.....	.....	1	1	5	4	11	4	7
4	0	0	0	0	2	6	16	17	16
Carbolic acid:									
1	0	1	1	7	.....	.....	6	4	5
2	4	8	10	9	16	13	13	.....	14

Results summarized in table 14 show that the virus is quite resistant to the action of these germicides. Copper sulphate was more effective in inactivation than any of the other materials tested, no infection being obtained from exudate treated for 2 hours in a concentration of 1:100. The leaf hoppers were able to pick up virus from exudate subjected to a 2-hour treatment with carbolic acid (1:25), bichloride of mercury (1:50), and formaldehyde (1:100), although infection was reduced at these concentrations. It is probable that carbolic acid and bichloride of mercury at a concentration of 1:25 had even less effect on the virus than is indicated by the number of infected plants. From the 1:25 concentrations of both carbolic acid and bichloride of mercury it was found difficult to remove enough of the germicide to avoid an effect on the leaf hoppers. Feeding was poor and the leaf hoppers were weak when they were removed from the medium and placed on plants, consequently it is improbable that the insects were as effective in picking up and transmitting the virus as they would have been had the medium on which they fed been more favorable.

INACTIVATION OF VIRUS BY PLANT JUICES

Severin and Freitag (*13*) showed that the expressed juice from Mammoth sweet corn mixed with juice from diseased beets inactivated the curly top virus in 2 hours. Results of tests described in this paper show that the virus in filtered beet juice and in beet juice to which alcohol was added was inactivated in 1 to 2 weeks, whereas, in filtered phloem exudate and in phloem exudate to which alcohol was added, the virus remained active 4 to 7 weeks. This evidence indicates that beet juice contains substances capable of inactivating the virus, though these inactivating substances are evidently not so effective in causing inactivation as those found in the juice of sweet corn.

To test further the effect of plant juices in inactivation of the curly top virus a number of species of plants ranging in susceptibility to curly top from very susceptible to very resistant have been selected. These tests were made with juice from Australian saltbush (*Atriplex semibaccata* R. Br.), nettle-leafed goosefoot (*Chenopodium murale* L.), tree tobacco (*Nicotiana glauca* Graham), cultivated tobacco (*N. tabacum* L.), yellow sorrel (*Oxalis corniculata* L.), tomato (*Lycopersicon esculentum* Mill.), alfileria (*Erodium cicutarium* (L.) L'Her.), chickweed (*Stellaria media* (L.) Cyr.), and susceptible and resistant beets (*Beta vulgaris* L.). Of these species Australian saltbush, nettle-leafed goosefoot, and tree tobacco are very resistant, and alfileria, chickweed, and the susceptible variety of beet are very susceptible. The other species are somewhat intermediate in susceptibility.

In making the tests, juice from the plants to be tested was mixed with juice from diseased beets in the proportion of 4 parts test juice to 1 part juice from diseased beets. The mixtures were placed in stoppered flasks and allowed to stand at room temperature. At intervals, 5 cc of each mixture was removed, added to 5 cc of 95-percent alcohol, and centrifugalized, and the supernatant liquid was discarded. The precipitate was washed once with 50-percent alcohol, dried, mixed with 1 cc of 5-percent sugar solution, and centrifugalized, and the supernatant liquid was used for testing for virus.

TABLE 15.—*Inactivation of the curly top virus by juices from different species of plants susceptible to curly top*

Source of juice	Plants inoculated	Plants infected by mixture of indicated age					
		Check [1]	2 hours	1 day	3 days	7 days	14 days
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Atriplex semibaccata	60	5	6	1	0	0	0
Chenopodium murale	60	13	13	25	23	13	1
Nicotiana glauca	40	21	17	16	14	5	0
Nicotiana tabacum	60	37	31	24	10	3	1
Oxalis corniculata (pH 2.3–2.5)	60	6	0	0	0	0	0
Oxalis corniculata (pH 6.8–7.2)	60	13	2	6	0	0	0
Lycopersicon esculentum	60	20	8	6	7	1	0
Erodium cicutarium	60	17	5	13	4	0	0
Stellaria media	60	9	12	21	4	0	0
Beta vulgaris (resistant)	60	21	23	20	26	13	1
Beta vulgaris (susceptible)	100	32	30	43	48	22	1

[1] Precipitation was made immediately after the juice from the test plant was mixed with juice from diseased beets.

The results of this series of tests are shown in table 15. Of the three most resistant plants the juice from Australian saltbush quickly inactivated the virus, whereas juice from nettle-leafed goosefoot and tree tobacco in general had no more effect than juice from beet. Of the three species probably most susceptible, the juice from alfileria and chickweed inactivated the virus in 7 days and juice from beet did not inactivate the virus in 14 days. These results are more striking in view of the fact that tree tobacco is a symptomless virus carrier (*2*), nettle-leafed goosefoot attenuates the virus (*3*), and chickweed restores attenuated virus to its maximum virulence (*12*).

Juice from yellow sorrel was the most effective of the juices in producing inactivation. Results in table 15 show that no virus was obtained from juice of this plant after 2 hours, and results of other tests not shown in table 15 indicate that the virus was inactivated in 30 minutes.

Juice from tomato and alfileria produced more rapid inactivation than juice from beet. Apparently juice from resistant beets is no more effective in producing inactivation than that from susceptible beets. It is obvious from these results that expressed juice from different species of plants varies greatly in its ability to inactivate the virus and that this variation is not correlated with resistance.

The question naturally arises as to how much of this apparent inactivation is due to an irreversible change in the virus itself and how much is due to an adsorption of virus by colloidal particles in such a way as to prevent its acquisition by leaf hoppers. It seems probable that juices from different species of plants vary in respect to the readiness with which virus is released from their alcoholic precipitates. For example, as compared with the addition of juice from healthy beets to the original virus-carrying medium, the addition of juice from tobacco increased and the addition of juice from chickweed and alfileria decreased the amount of virus recovered from the alcoholic precipitates. Since this result was evident in the short-time exposures and since the virus remained active in these mixtures for 3 or more days, it seems probable that much of the variation in infection from preparations made during the first 2 hours of aging of the juices may be the result of variations in completeness of release of the virus from colloidal material into a supernatant liquid. However, the rapid inactivation by juice from yellow sorrel and the more gradual loss of virus in other juices indicates that there are factors present that are capable of causing complete and permanent inactivation.

The source of the inactivating materials present in plant juice has not been determined definitely. Severin and Freitag (*13*) found that virus apparently remained active for a longer time in beet juice from which air was partially excluded than in juice exposed to the air, the implication being that virus is inactivated by products of oxidation in expressed juice. However, the rapid inactivation by certain plant juices indicates that there are substances other than products of oxidation that are destructive to the virus. Also the evidence (*2*) indicating the occurrence of little or no virus in parenchyma tissues points to the occurrence of substances in parenchymatous cells that either inactivate the virus or prevent its multiplication. It seems probable that at least a large share of the inactivating substances present in plant juices are derived from living parenchyma cells.

If the inactivating substances are derived from parenchyma, the lack of correlation between the apparent concentration of inactivating substances in expressed juice and resistance does not necessarily eliminate the inactivating substances as factors in resistance. Since the virus is concentrated in the phloem, it is probably not greatly influenced by inactivating materials that occur only in the parenchyma. However, if inactivating substances pass from the parenchyma into the phloem their effect on the virus and perhaps their importance in resistance may depend on the concentration reached in the phloem. Under such conditions the permeability of the cells surrounding the phloem to inactivating substances might be more important in determining resistance than the total concentration of inactivating materials in the plant.

The chemical nature of substances in plant juices responsible for virus inactivation, especially those present in the juice of yellow sorrel and Australian saltbush, offer an interesting field for investigation.

At present very little is known about the character of these substances and any statements that are made on this subject must necessarily fall more or less in the realm of speculation. However, since the virus is quickly inactivated at a reaction of pH 2.9 and lower and is apparently somewhat sensitive to acid reactions, it is suggested that acidity of the expressed juice may have an inactivating influence in some instances. This would apply especially to yellow sorrel, since the reaction of expressed juice from this plant ranges between pH 2.0 and 2.3 and when mixed with beet juice in the proportion of 4 parts yellow sorrel juice to 1 part beet juice, the reaction of the mixture ranges between pH 2.3 and 2.5. However, when this juice was adjusted to a reaction of pH 6.8 to 7.0 it was still capable of inactivating the virus, though inactivation was much less rapid than when the reaction was not adjusted. It seems probable that expressed yellow sorrel juice contains two factors, each capable of inactivating the virus; the more effective of these factors is acidity and the less effective is the inactivating substances remaining when acids are neutralized.

Substances not acid in character are probably responsible for inactivation in juices from the remainder of the plants tested. With the exception of juice from alfileria, which has a reaction of pH 5.2 to 5.6, the juices from these plants have about the same degree of acidity as beet juice, ranging from pH 5.8 to 6.8. It does not seem probable that this range of acidity could account for the wide range in ability to inactivate the virus, especially since there is no correlation between acidity and rate of inactivation.

DISCUSSION

The physical and chemical properties of the curly top virus, so far as they have been determined, are similar in many respects to the properties that are considered typical of plant viruses as a group. However, the curly top virus has certain characteristics that are specific. Among the characteristics that serve to distinguish it from other viruses may be mentioned an extreme resistance to the deleterious action of alcohol and acetone and an activity in a range of hydrogen-ion concentrations that extends far toward the alkaline side of the scale.

The available information affords no definite clue as to the reason for prolonged periods of activity of the curly top virus in alcohol and acetone. The elucidation of this problem must await a more complete understanding of the influence on the virus of certain chemical and environmental factors. However, certain evidence is available that may have a bearing on the reaction of the virus to hydrogen-ion concentration. As compared with other viruses that have been studied in this connection, a greater sensitivity to acid reactions and a greater tolerance to alkaline reactions might be expected on the basis of the relation of the virus to the plant and to the insect vector.

In the beet plant the virus is rather definitely known to be located in the phloem. The tissues or fluids associated with the retention of the virus by the beet leaf hopper have not been determined completely but it is quite evident that the virus passes from the leaf hopper to the plant through the medium of the saliva. Evidence

not yet published indicates that the virus is present also in the body fluid. In view of this evidence and of the fact that Storey (*15*) has shown that the virus of maize streak occurs and multiplies in the body fluid of its leaf hopper vector, *Balclutha mbila* Naude, it seems probable that the body fluid may be the chief reservoir of the curly top virus in the beet leaf hopper.

The hydrogen-ion concentration of the various tissues and fluids of the plant and insect vector in which virus occurs has been determined within certain limits. Natural exudate on beet petioles and small drops of exudate that appear immediately above the ends of the veins on the cut surface of beet roots are alkaline in reaction. Physiologists have shown for a number of plants that the phloem content is alkaline, and it is safe to assume that the reaction of the phloem content of beet is well above pH 7.0. Fife (*10*) has shown that the salivary secretions in the beet leaf hopper are distinctly alkaline. Determinations [4] made on the body fluid have given values ranging from pH 7.0 to 7.6. Thus, with the possible exception of a period in the alimentary tract of the insect following ingestion of plant substance taken up in feeding, the virus normally exists in a medium alkaline in reaction. The virus apparently has a greater tolerance to alkaline compounds than that possessed by viruses that occur naturally and increase in the acid cell sap of parenchymatous tissue.

Certain results of the studies on properties may be interpreted as having a bearing on the relation of the virus to the sugar beet plant. For example, the results of the studies on dilution clearly indicate that less virus is available from expressed beet juice than is available from phloem exudate. This is shown both by the number of plants infected and by the maximum dilutions from which virus was obtained. These results probably reflect a difference in initial concentration of virus in the two types of material. Considering the experiments in which only one leaf hopper was used on each test plant, the maximum dilutions from which virus was obtained were 1:1,000 for phloem exudate and 1:300 for expressed beet juice. Thus it might be reasoned that the concentration of virus in phloem exudate is more than three times that in beet juice. On the basis of infection obtained from the different dilutions of material tested, assuming that 0.1 g of leaf hoppers is equal in volume to 0.1 cc, the virus content of the beet leaf hopper is slightly less than that of phloem exudate but considerably greater than that of beet juice. The results with different dilutions of phloem exudate and beet juice constitute additional evidence to support the concept that virus in the beet plant is concentrated in the phloem. The virus found in expressed juice may be derived from material that has passed from the phloem into the intercellular spaces of surrounding tissues or from phloem content that escapes and becomes mixed with sap from other tissues during the process of extraction.

Since so little is known regarding the factors responsible for the close association of the curly top virus with the phloem, and its partial or practically complete restriction to this tissue, certain of the results of these studies are of interest as having a bearing on this subject. In any limitation of virus to phloem there are two possibilities that must be considered: (1) Tissues that surround the phloem

[4] These determinations were made in collaboration with J. M. Fife, biochemist, Division of Sugar Plant Investigations, Bureau of Plant Industry. Details of this work have not been published.

may tend to prevent passage of virus into or out of the phloem, and (2) virus that gains entrance to parenchymatous tissues may remain active but fail to multiply or it may be inactivated. The permeability factor may be important in limiting the quantity of virus that gains entrance to parenchyma, but this factor alone can hardly account for the virus distribution and concentration that has been shown to occur in different tissues of the plant. Virus probably gains entrance to parenchyma in several ways. It is possible that it passes directly from the phloem into the surrounding parenchyma cells, and it is undoubtedly introduced into parenchyma cells by artificial inoculation and by leaf hopper feeding. However, there is no evidence that increase of the virus in parenchyma follows its introduction by any of these methods. In view of these facts it is logical to conclude that parenchyma tissue is unfavorable for virus multiplication and that certain constituents of parenchyma cells may be capable of inactivating the virus.

Results of experiments with expressed plant juices show that substances capable of causing rapid inactivation occur in the juice of yellow sorrel and that substances capable of causing less rapid inactivation occur in the juice of beet and several other plants. If these inactivating substances occur also in the sap of living cells, as seems probable, they may be the factors chiefly responsible for the partial or practically complete restriction of the virus to the phloem. If their behavior in the living cell is comparable to their behavior in expressed juice, their effectiveness would vary with the species of plant involved. Practically complete restriction of virus to the phloem might be expected in yellow sorrel and Australian saltbush, and less complete restriction might be expected in such plants as beet and tobacco.

SUMMARY AND CONCLUSIONS

Because of the difficulty of obtaining infection by artificial inoculation and the necessity of using the beet leaf hopper for determining the presence of virus in any particular medium, property studies of the curly top virus have required the development of a technic for utilizing the vector for transmission experiments, which has not been necessary in similar studies with viruses that more readily produce infection after artificial inoculation. The first material from which artificially fed leaf hoppers were induced to acquire virus was beet juice. However, this proved to be either toxic or unacceptable to the beet leaf hopper and is not a very satisfactory material for use in property studies. Two types of material that have a relatively high virus content and are more acceptable to the beet leaf hopper have been found. Of these two materials the more satisfactory is the phloem content of diseased beet plants. This can be obtained in small quantities as natural exudate from the beet petioles and leaves and in larger quantities from the cut surface of diseased beet roots. The second type of material was prepared by separating the virus in beet juice from the materials toxic or unacceptable to the beet leaf hopper. In effecting this separation the precipitate produced upon the addition of alcohol is thrown down by centrifugalization, washed once with 50-percent alcohol, dried, made up to the original volume of juice with 5-percent sugar solution, and centrifugalized. The supernatant liquid of the second centrifugalization contains considerable virus and is a favorable food for the beet leaf hopper.

This method of preparing a virus-containing medium can be used with phloem exudate, suspensions of crushed beet leaf hoppers, or other materials containing proteins. Acetone may be used instead of alcohol without appreciably influencing the result. The method is valuable in separating virus from materials that are toxic or unacceptable to the beet leaf hopper, especially in experiments involving the treatment of the virus with various chemical compounds.

The removal of the virus from beet juice and phloem exudate by the precipitate formed upon the addition of alcohol is practically complete. The release of the virus from the precipitate upon the addition of 5-percent sugar solution is only partial, since considerable virus was obtained from each of several successive washings of precipitate from phloem exudate and from three successive washings of precipitate from beet juice.

When the materials just described were used as sources of virus it was found that virus concentration in phloem exudate was considerably greater than that in beet juice and somewhat greater than that in the beet leaf hopper. The dilution of phloem exudate from which virus was recovered apparently depended somewhat on the number of artificially fed leaf hoppers used to inoculate each seedling beet. Virus was recovered from dilutions of 1 : 1,000 in experiments in which 1 artificially fed leaf hopper was placed on each plant and from dilutions of 1 : 20,000 in experiments in which 10 leaf hoppers were placed on each plant.

The common filters, such as the Berkefeld V, N, and W and the Mandler medium and fine grades, do not remove the virus from phloem exudate, water suspension of alcoholic precipitate of leaf or root juice, or from beet leaf juice. With the exception of leaf juice, the recoverable virus in the liquids after passing through these filters was about as great as in the unfiltered original liquid.

The resistance of the virus to aging in a liquid medium depends considerably on the medium in which the virus is preserved and perhaps somewhat on the presence or absence of micro-organisms. In filtered and unfiltered beet leaf juice virus was recovered after 7 days. It was recovered from unfiltered water wash of alcoholic precipitate of leaf juice after 14 days and from filtered water wash of alcoholic precipitate of leaf juice after 28 days.

The resistance of the virus to desiccation also depends somewhat on the medium in which it is kept. Virus remained active 10 months in dried phloem exudate, 5 months in alcoholic precipitate of phloem exudate, 4 months in dried beet tissue, 2 months in alcoholic precipitate of beet leaf juice and beet root juice, and 6 months in dried beet leaf hoppers.

Phloem exudate subjected to a temperature below freezing for a period of 18 months showed no great loss in virus content. Daily alternate freezing and thawing for a period of 3 weeks did not appreciably lessen the virus content of diluted phloem exudate or that of the water wash of the alcoholic precipitate of leaf juice. The thermal inactivation point of the virus lies between 75° and 80° C. A 10-minute exposure to a temperature of 76° to 79° attenuated the virus.

No virus was recovered from liquids having a pH value of 2.9 or lower. Alkaline reaction of the medium at least as high as pH 9.1 did not inactivate the virus in 2 hours and, in most instances, the alkaline reactions probably had little or no deleterious influence.

Evidence is presented indicating that the virus normally occurs in an alkaline medium in both the plant and insect vector. This may account in part for the resistance of the virus to inactivation by alkaline compounds.

A 2-hour treatment with 90-percent alcohol and with lower concentrations had no appreciable effect on the virus. Absolute alcohol reduced but did not destroy activity in a 2-hour period. A 2-hour treatment with acetone had no apparent effect at any concentration. Virus was active after 56 days in 50- and 75-percent alcohol and in 75-percent acetone, although activity had been considerably decreased. The virus also exhibits considerable resistance to the lethal action of a number of common disinfectants, active virus being recovered from copper sulphate (1:200), bichloride of mercury (1:50), formaldehyde (1:100), and carbolic acid (1:25).

The expressed juice from beet and a number of other species of plants is able to cause inactivation of the curly top virus in periods ranging from 30 minutes to more than 14 days, depending on the species of plant from which the juice is derived. The time required for inactivation by juice from different species of plants apparently is not correlated with the degree of resistance of the species. Since the curly top virus is believed to be more or less closely restricted to the phloem, it is suggested that if the inactivating substances in expressed juice are derived directly from the plant, as seems probable, they occur in the parenchymatous tissues. If they have any effect in producing resistance this effect is probably governed by the degree to which they are able to diffuse into the phloem. Inactivating substances of the parenchyma may function in limiting virus to the pholem tissue.

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PRODUCTIVITY OF THE CAMPHOR SCALE AND THE BIOLOGY OF ITS EGG AND CRAWLER STAGES[1]

By C. I. Bliss, formerly *entomologist*, A. W. Cressman, *associate entomologist*, and B. M. Broadbent, *assistant entomologist, Division of Fruit Insects, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

The eggs of the camphor scale (*Pseudaonidia duplex* Ckll.) are laid beneath the scale covering of the mother, and there they complete their development. Upon hatching they are known as "crawlers." After an indeterminate time the crawlers emerge and crawl about over the host plant until they settle and begin feeding. The present studies are concerned with this critical phase of the life history, during which the maximum abundance and distribution of the succeeding generation are determined. The later development of the scale is discussed in another paper.[2]

PRODUCTIVITY

DETERMINED FROM EMERGENCE IN THE LABORATORY

The productivity of the camphor scale cannot be studied directly without removing the scale covering that conceals the eggs, but that of undisturbed females can be estimated indirectly. Small potted camphor-trees and Satsuma orange trees on which there were one or more mature females were examined periodically, and all crawlers and newly settled young were recorded and removed. In some cases the observations were as frequent as every 3 hours during the day, in others at intervals of 2 or 3 days; hence these records exclude a variable number of crawlers that emerged and died between observations. Furthermore, the natural variability in productivity may have been augmented by differences in the potted plants and in their position in the insectary. For these reasons the plants on which there were more than an average number of offspring per female may sometimes be the best index to productivity in the field.

Table 1 shows the productivity of 24 second-brood females on 1- and 2-year-old camphor-tree seedlings. Six plants were examined at intervals of 1 or, less often, 2 days, beginning August 7 or 8, 1930. These dates did not necessarily represent the beginning of production for all the females on the plant, as there may have been a difference of several days in this regard. With one practically infertile exception (no. 31), there was a marked falling off in the daily production per female in the successive 10-day periods, and 50 percent of the progeny emerged in the first 6 to 13 days. At this season of the year the mean temperatures are nearly maximal. The average number of offspring per female was 110.

[1] Received for publication Nov. 5, 1934; issued April 1935. These studies cover one phase of the investigations at the laboratory formerly maintained by the Division of Fruit Insects of the Bureau of Entomology at New Orleans, La. Prior to 1926 the experiments were conducted by T. F. Catchings, and H. K. Plant was in charge of the laboratory.

[2] Cressman, A. W., and Bliss, C. I., with the assistance of Kessels, L. T., and Dumestre, J. O. Biology of the camphor scale and a method for predicting the time of appearance of stages in the field. Jour. Agr. Research 50: 267-283, illus. 1935.

TABLE 1.—*Productivity of undisturbed second-brood females of Pseudaonidia duplex on 1- and 2-year old camphor-tree seedlings, in the insectary, 1930*

Plant no.	Date production started	Median date of production	Date production ended	Length of production period		Producing females	Total progeny	Average progeny per female	Average progeny per female per day in successive 10-day periods				
				To median date	Entire period				First	Second	Third	Fourth	Fifth
				Days	*Days*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
30	Aug. 7	Aug. 18	Oct. 1	11	55	5	839	168	7.3	4.3	2.3	1.6	0.7
31	Aug. 8	Aug. 10	Aug. 13	2	5	1	12	12	1.0	.2			
32	Aug. 7	Aug. 19	Sept. 25	12	49	6	524	87	3.8	2.7	1.2	.6	.2
33	Aug. 8	Aug. 16	Sept. 4	8	27	2	357	178	10.4	6.0	1.2		
34	Aug. 7	Aug. 20	Oct. 11	13	65	4	650	162	6.8	3.9	2.4	1.9	.4
35	Aug. 8	Aug. 14	Oct. 9	6	62	6	266	44	2.9	.4	.3	.3	.1
Mean temperature (° C.)									27.3	26.0	25.8	25.9	23.9

In 1923, 13 first-brood females on 5 camphor-tree seedlings produced on an average 125 offspring per female and from 81 to 185 per plant. Productivity on Satsuma orange in 1924 compared favorably with that on camphor-trees, as is shown in table 2. In this case the newly emerged and settled young were removed daily or oftener.

TABLE 2.—*Productivity of undisturbed Pseudaonidia duplex females on Satsuma orange, budded stock in insectary, as determined by counts of progeny, 1924*

Brood of females	Plant no.	Date production started	Median date of production	Date production ended	Length of production period		Producing females	Total progeny	Average progeny per female
					To median date	Entire period			
					Days	*Days*	*Number*	*Number*	*Number*
First	1-10	June 15	June 23	Aug. 6	8	52	18	3,698	205
	1-11	June 17	June 24	Aug. 4	7	48	26	3,659	141
	1-13	June 15	June 26	Aug. 8	11	54	35	4,609	132
	1-16	do	June 25	Aug. 4	10	50	13	2,847	219
	1-17	June 16	June 30	Aug. 11	14	56	23	2,708	118
	1-18	do	June 25	Aug. 6	9	51	28	3,287	117
	1-19	June 17	July 4	Aug. 9	17	53	7	931	133
	Average				10.9	52.0			145
Second	2-3	Aug. 17	Sept. 7	Oct. 21	21	65	4	609	152
	2-5	Aug. 18	Aug. 28	Oct. 17	10	60	7	513	73
	Average				15.5	62.5			102

The possible multiplication during two generations in a single season was determined by counting the number of scales removed from each of three potted camphor-trees. By June 25, 1924, 83 scales had developed to maturity on these plants and, judged from other experiments on the same brood, about two-thirds of them may have produced offspring. As leaves dropped off from time to time, their scale populations were counted, and 8 months later, February 23–25, 1925, the number remaining on each plant was recorded. These 83 females accounted for 34,404 scales, or 415 per female. Because premature dropping of leaves through the season reduced the number of females that might normally have reproduced, this number represents a minimum rather than a typical case.

The potential progeny in an entire season in the absence of crowding, such as occurred in the preceding experiment, and without attack by parasites or predators, has been estimated from insectary experiments on 5,077 scales in 1930, covering the different parts of the life cycle. It was assumed that an average of 100 of the progeny of each female would settle successfully, and one-half of them would be females. Of these 50 females, 16.7, or 33.4 percent, would complete development and reproduce. If the same reproductive rate continued through all three generations, from 1 fertile overwintering female there would be 4,657 reproducing females the following year.

DETERMINED FROM EGGS FOUND BENEATH FIELD-COLLECTED ADULTS

Periodic counts of eggs from field-collected adults indicated that the beginning of oviposition antedated the first appearance of crawlers by from 1 to 5 weeks, depending primarily upon the time of the year and the brood. In 1926 weekly counts were made of the scale populations on camphor-tree twigs collected in Audubon Park, New Orleans. A few females laid eggs during the winter, but spring oviposition did not start until February 9, when 6 percent of the surviving females were found to be producing. Eggs had hatched beneath 5 percent of the producing females on March 16, 5 weeks later, but hatching was not general until March 30, when there were crawlers under 24 percent of the ovipositing females. Practically none of the young settled until April 6, 8 weeks after spring oviposition had started.

The long embryonic period favored the accumulation of eggs under the scale covering. From groups of individuals estimated to have started production at the same time the number of eggs increased from an average of 4.3 per female on February 9 to 64.5 (11 females, range 58 to 74) on March 23 and 81.5 (5 females, range 74 to 88) on March 30. Crawlers tended to remain under the parent scale covering for some time after hatching; under one scale there were 32 of them in addition to 48 eggs on April 6. The percentage of dead adults varied but little until May 11, when there was a marked increase. If these are assumed to be the first individuals of the overwintered females to oviposit, they laid eggs over a period of about 13 weeks, although no crawlers appeared during the first 8 weeks.

The oviposition and incubation periods during the summer were much shorter. Egg deposition by adults of the first generation in 1926 had started by June 15 in 9 percent of the females, with an average of 10 eggs each. A week later more than 60 percent had reproduced, females of comparable age showing an average of 30 eggs apiece and a few newly hatched crawlers. Many eggs had hatched, and the crawlers had emerged and settled by June 29, 2 weeks or less from the beginning of production as compared with 8 weeks in the spring. The number of eggs under each female never equaled the number found under the overwintered females, indicating that the higher summer temperature increased the rate of embryonic development more than the rate of oviposition. The first adult females of the first generation died July 20, 5 weeks after the first eggs appeared.

In 1926 both newly hatched crawlers and newly settled first-instar nymphs were found on August 17, 1 week after the first eggs were laid by second-brood females. The maximum number of eggs recorded from a single female was 63 as compared with 62 for the first-brood females and 88 for the overwintered females. The increased mortality

on September 21 showed the average reproductive period for the first individuals of the second brood to be about 6 weeks.

The span of productivity of females in the field was somewhat less than that of undisturbed females infesting potted camphor-trees (table 2). The longer interval in the insectary can probably be attributed to overlapping periods of maturity in the various females on each plant.

DETERMINED FROM OVIPOSITION IN THE LABORATORY

Productivity can be studied at closer range than by either of the two foregoing methods. When the scale coverings were removed with sufficient care from field-collected adults, it was possible to observe oviposition directly. For this purpose short sections of camphor-tree twigs bearing females were placed in Petri dishes, in the tops and bottoms of which blotting paper had been fitted, with the ends of the twigs embedded in moist sand at one edge of the dish. In this way conditions of humidity and darkness similar to those beneath the female covering were obtained, and the twigs remained green over most of the oviposition period. Freshly extruded eggs usually adhered to the posterior tip of the exposed female and were removed with a fine moistened brush at intervals of from 4 to 24 hours. The eggs removed from each female at each examination were kept separately in order that the time of hatching might be determined.

Effect of Temperature on Productivity

Temperature influenced the rate of oviposition more than any other factor. At constant temperatures egg laying was distributed equally throughout the 24 hours. Under fluctuating outdoor temperatures, however, fewer eggs were laid at night than during the day, especially in the spring, and in 2 out of 3 series of observations fluctuations followed changing temperatures rather closely.

In 1929 the production of 31 first-brood females kept at a constant temperature of 28° C. and of 15 first-brood females kept at 20° C. was recorded for 16 successive 6-hour intervals. The females kept at 28° laid on an average 2.42 ± 0.04 eggs in 6 hours, and those kept at 20° laid on an average 1.18 ± 0.04 eggs, as indicated in figure 1.

In 1926 second-brood females and in 1931 overwintered females were placed in Petri dishes in the thermograph shelter in the insectary, and records were kept of their production. After abnormal individuals and those that died prematurely had been eliminated, the average production per female in 12 hours was plotted against the temperature (fig. 1). The records for the second-brood adults were based on 41 females initially, but after 5 days this number was reduced to 19. The eggs were removed every 4 hours, at 1, 5, and 9 o'clock and for comparative purposes the record for each 4-hour interval was multiplied by 3. The records for the overwintered females were based on from 13 to 20 individuals and were taken at 9 a. m. and 9 p. m. A rise in temperature increased the rate of egg deposition in a fairly consistent manner, especially between 14° and 29° C. By the method of partial averages straight lines have been fitted to the points between the parallel broken lines in figure 1. Below 14° there were many cases in which production was less than expected, and in five instances it stopped altogether. Temperatures above 29° also

retarded egg laying. This optimum temperature is considerably below temperatures frequently encountered in New Orleans.

Females fr m the first brood of 1931 were exposed to the same range of temperature as those of the second brood of 1926, but, although the average rate of laying was nearly as high as in the earlier series, the variation from one examination to the next showed no correlation with temperature.

Other Factors Affecting Productivity

Other factors that might have affected the rate of oviposition were of minor importance in these experiments. The age of the females, as judged by the number of eggs and crawlers originally found under

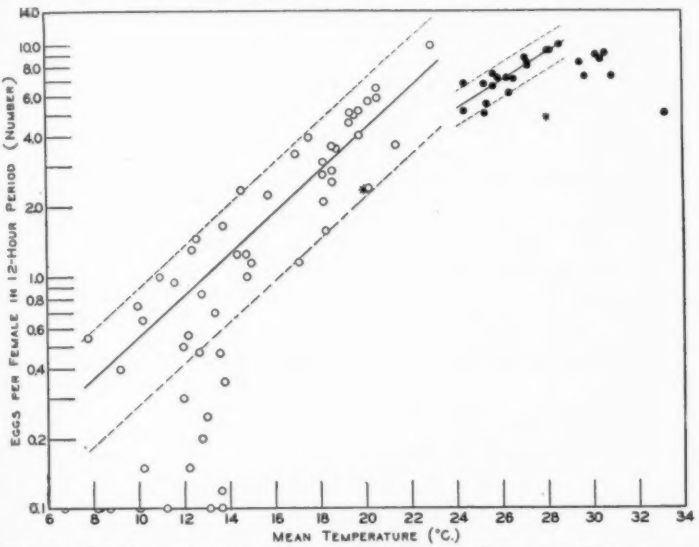

FIGURE 1.—Effect of temperature upon rate of oviposition of *Pseudaonidia duplex*. Solid circles represent records of the second-brood females in 1926, empty circles represent those of the overwintered females in 1931, and asterisks (*) represent those of the second-brood females in 1929 at two constant temperatures, 20° and 28° C. The constant-temperature records have been multiplied by 2 for plotting purposes. Half circles below the base line represent zero records.

the scale covering, had no influence on the number of offspring produced during the second and third days of the experiment, when production should have been nearly normal. The manipulation necessary to remove the eggs stimulated egg laying slightly. Females kept at a constant temperature of 28° C. in August 1929 and examined at intervals of 6, 12, and 24 hours laid on an average 10.2 ± 0.2, 9.8 ± 0.4, and 9.0 ± 0.03 eggs, respectively, per 24 hours.

The age and size of the woody tissue on which the females had settled had a significant effect on production in the experiment started on August 24, 1926. Some of these females were on slender new growth, whereas others were on older twigs of twice the diameter. On August 27 no difference (0.04 ± 0.11 egg) was found in the average productivity over 4-hour periods, but on the following 3 days those

females on the younger wood laid on an average 1.07 ± 0.07 more eggs every 4 hours than did those on the older wood.

Females from which the scale covering had been removed would not be expected to produce a full quota of eggs, if only because of the shortened span of productivity. Nevertheless, in these experiments many of the adults laid more eggs than the average produced by the undisturbed individuals shown in table 2. Under a constant temperature of 28° C. and with examinations at intervals of from 6 to 12 hours, 22 females that had averaged 39 eggs each before the scale covering was removed laid a total of from 94 to 263 eggs, averaging 158.4 ± 4.4, before they died or the experiment was terminated. The length of the oviposition period ranged from 9 to 19 days after the females were placed under observation. Under fluctuating temperatures 16 overwintered females just beginning production laid from 71 to 230 eggs, averaging 151, in from 38 to 50 days (average 45.6), whereas 8 females of comparable age of the next generation produced from 72 to 113 eggs, or a mean of 92.7, in from 6 to 13 days (average 9.4).

EGG STAGE

In August 1926 two experiments were conducted to obtain data on hatching and the length of the egg stage of the camphor scale. In the first experiment, each day for 8 successive days field-collected females were examined and the eggs placed on moist paper in Petri dishes, the eggs removed from each female at each examination being kept separately. The eggs from some females hatched much sooner than those from others, but all the eggs laid on a single day by each adult completed development in nearly the same time.

On the basis of similarity in the length of the developmental periods of their eggs, the 21 females isolated on August 6 could be grouped into 5 types. The mean length of the incubation period of eggs laid by each type of female on each day of the experiment is shown in figure 2. In eggs laid by females of types 1 to 3 from 6 to 8 days were required for postuterine development at the beginning of the experiment and after that the time decreased gradually. In eggs laid by females of types 4 and 5 the developmental period was comparatively brief for the first 5 days and then became slightly longer. For each type the total variation, as measured by the standard deviation, was small and about equal for eggs laid on any 1 day. Because of large differences between the means, the coefficient of variation, which usually measures inherent variability, varied enormously. For eggs laid on August 9 it ranged from 5 to more than 160 percent (table 3).

TABLE 3.—*Statistical constants for length of incubation period of eggs laid by five types of Pseudaonidia duplex females on Aug. 9, 1926*

Type of female	Mean length of egg stage	Standard deviation	Coefficient of variation	Type of female	Mean length of egg stage	Standard deviation	Coefficient of variation
	Days	*Days*	*Percent*		*Days*	*Days*	*Percent*
1	8.13	0.40	4.9	4	1.39	0.56	40.3
2	6.67	.47	7.0	5	.28	.45	160.7
3	4.95	.59	11.9				

The percentage variation in the rate of development in type 1, and possibly type 2, is about normal for homogeneous biological material—an indication that the eggs were deposited soon after they began to develop. Since the standard deviation is practically constant, differences in the length of the incubation period for eggs from the last

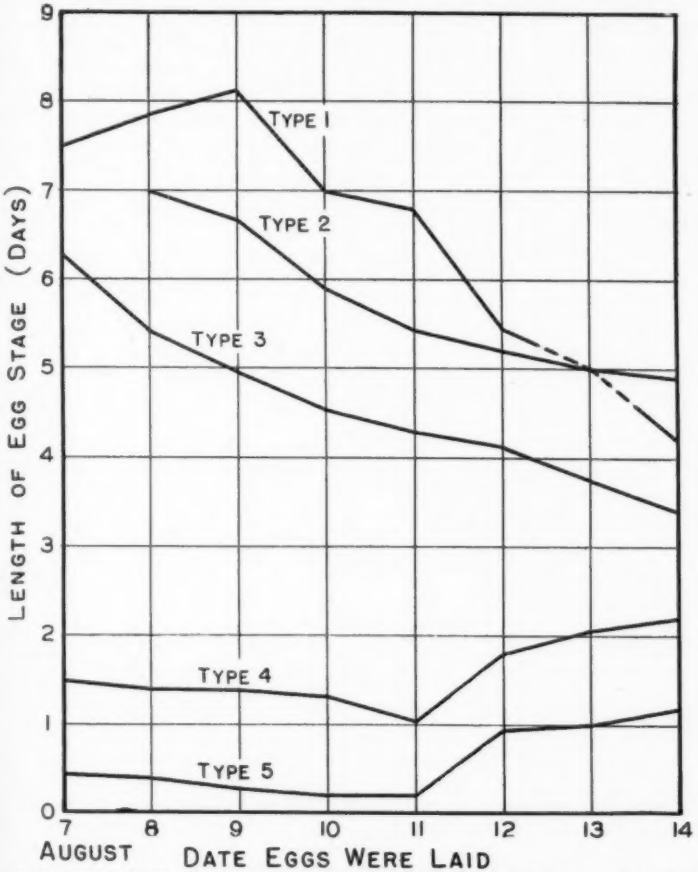

FIGURE 2.—Average length of incubation period of eggs removed from each of five types of field-collected *Pseudaonidia duplex* females, August 1926

three types of females may be due to differences in the length of time they were retained in the parent's body before deposition. Diversity in age was later proved to be the most likely explanation. The two groups that laid eggs about ready to hatch were identified as the last of the first brood, while the other three groups were probably newly mature adults of the second brood, as indicated by the presence of females of the two broods in contemporary seasonal-history counts and by the rapid decrease in the length of the incubation period of the eggs of the younger females under mean daily temperatures of 28.0 ± 0.2° C.

On August 24 the experiment was repeated, with one modification in procedure. When the scale covering was removed from each female, the number of eggs and crawlers beneath it was recorded, and also the absence or abundance of eggshells and the presence of progeny settled beneath the parent scale. The relative age of each female, as judged from these data, was then recorded in terms of the total number of eggs laid on or before August 24. Since at this time females of the second brood had been laying for about 2 weeks, individuals of all ages were available. The length of the developmental period of eggs laid subsequently was then determined as before. The 51 females used in the experiment were then divided into 6 age groups as measured by production prior to the beginning of the experiment, and for each group the average length of the egg stage was calculated for eggs laid on the first day of the experiment and for eggs laid during the first 4 days of the experiment. The results are given in table 4.

TABLE 4.—*Length of egg stage of Pseudaonidia duplex related to production prior to the beginning of the experiment on Aug. 24, 1926*

Eggs produced on or before Aug. 24		Females	Mean length of egg stage for eggs laid—	
Range	Mean		On first day of experiment [1]	On first 4 days of experiment [2]
Number	*Number*	*Number*	*Days*	*Days*
0–10	3.8±0.5	17	6.86±0.12	6.09±0.13
11–20	17.6± .8	5	5.84± .02	4.99± .43
21–40	32.7±1.5	7	4.37± .15	4.02± .29
41–60	49.2±1.4	11	3.47± .17	3.22± .18
61–80	69.2±2.1	5	3.50± .21	2.88± .11
81–100	86.8±1.4	6	2.07± .22	1.96± .21

[1] Averages for individual eggs.
[2] Averages of means for each female.

There was a close correlation between the production age of the female and the length of time fertilized eggs were held before being deposited. This was demonstrated both by the shortening of the egg stage as the female aged and by the fact that, for females of the same age group, the average for the first 4 days was in every case less than that for the first day only. After eggs had begun to hatch and crawlers to emerge, the previous production of a female could be determined only approximately, an uncertainty that applied particularly to the oldest group. Oviposition probably started soon after the first eggs began developing, as judged from the 10-percent coefficient of variation in the length of the egg stage for eggs laid by the youngest group of females.

In 1931 eggs were removed from overwintered females and females of the first brood that were just beginning to reproduce. As in previous experiments, the females could be grouped into a small number of classes on the basis of similarity in the length of the egg stage. Observations on overwintered females were started February 25, with egg removals at 12-hour intervals. The average length of the incubation period of eggs from 9 representative females has been plotted in figure 3, together with that of eggs from 2 females that varied most from the average. Since the temperatures increased

from the beginning to the end of the experiment, the shortening of the egg stage was due to a rising temperature as well as to an increase in the degree of embryonic development prior to oviposition.

Observations on the first-brood females were begun on June 24, with egg removals at 4-hour intervals. Although of nearly the same production age, these females showed a variation of nearly 2 days in the length of time eggs were retained before being deposited. As in the earlier experiments, the later eggs hatched in a much shorter time than the first ones to be laid.

Most of the eggs laid on a single day by a given female hatched within a short period, but there were some exceptions. Occasionally

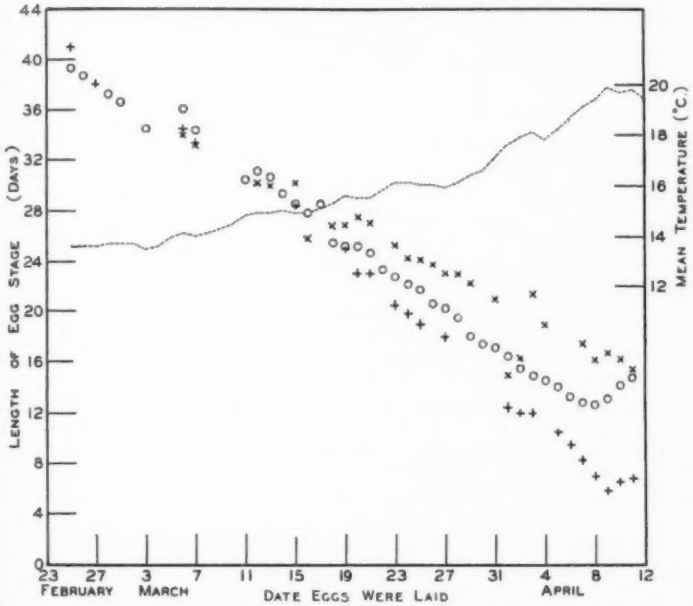

FIGURE 3.—Average length of incubation period of eggs of *Pseudaonidia duplex* laid by overwintered females in 1931. The circles represent averages of eggs from 9 typical females: the two series of crosses (+ and ×), eggs from the 2 females, in a total of 23, which varied most widely. The temperatures, represented by the dotted line, are the means for the incubation period of typical eggs hatching at each observation.

eggs adhered in strings as they were laid. In removing these care was taken to preserve the order of laying, so that it might be compared with the order of hatching. In general, the eggs hatched in the order in which they were laid, but some eggs hatched from 12 to 24 hours before older ones in the string. In 1931 the incubation period of a few eggs differed by 2 or 3 days from that of the majority of the eggs laid on the same day. Some of these exceptional eggs had apparently just begun to develop when deposited; others were laid in such an advanced stage that they hatched during the 4-hour interval between egg removals.

Because of the influence of the age of the parent, only the first eggs to be laid after removal of the coverings were deposited sufficiently early to permit measuring the effect of temperature upon the rate of

embryonic development. To determine this factor, observations were made on the length of the developmental period of such eggs taken from females of each brood, thus representing three different seasons of the year. The results have been summarized in table 5. When the mean temperature of the incubation period was 13.6° C., an average time of 39.6 days was required to complete development, whereas the eggs hatched in 6.8 days under a mean temperature of 27.1° C.

TABLE 5.—*Effect of temperature on the length of the developmental period of eggs of Pseudaonidia duplex laid on the first day of observation by females that had laid previously an average of 3.6 eggs*

Date eggs were laid	Brood of females	Females	Eggs	Mean temperature during incubation	Length of egg stage			
					Mean	Minimum	Maximum	Coefficient of variation
		Number	*Number*	*° C.*	*Days*	*Days*	*Days*	*Percent*
Aug. 25, 1926	Second	17	70	26.9	6.86±0.12	5	8	10.3
Feb. 25, 1931	Overwintered	8	8	13.6	39.62± .18	39	41	1.8
June 24, 1931	First	11	25	27.1	6.82± .04	6.2	7.5	4.7

The mortality of eggs removed to Petri dishes ranged from 10 to 20 percent in warm weather. Under the low temperatures of early spring many more were killed by fungi, a thing that did not occur in the field. Eggs placed in open Petri dishes and kept in closed incubators with fan circulation failed to hatch, but when fresh air was introduced continuously by a filter pump, hatching compared favorably with that in the insectary. Mortality in the field could be estimated approximately from the number of dead eggs and crawlers under spent females. In the first and second broods the mortality was probably less than 5 percent of the total production, but in the third brood the mortality of eggs, and especially that of crawlers, was much greater.

EMERGENCE AND SETTLING OF THE CRAWLER

EMERGENCE

The newly hatched crawler did not leave the protection of the parent scale immediately, but was responsive to external conditions. This was indicated particularly by the diurnal variation in the number emerging. In August 1923 potted camphor-tree plants with undisturbed reproducing females were examined at 6 and 9 a. m., 12 m., and 6 p. m. for several days, at which times all crawlers and newly settled first-instar nymphs were removed. Crawlers seldom emerged during the night, although a few newly settled individuals with white covers were found at 6 a. m. It is probable that these had appeared the preceding day but had been overlooked, as in a study of night emergence during the summer of 1925 no crawlers were found to emerge during the night even when the plants were illuminated with electric lights. From 6 a. m. to 6 p. m. the rate of emergence varied considerably, as is indicated in table 6. Usually more than half the crawlers left the parent scale covering between 9 a. m. and noon, although the proportion varied from day to day.

TABLE 6.—*Number of crawlers of Pseudaonidia duplex emerging from beneath undisturbed females on potted camphor-trees in different intervals during the day, August 1923*

Females	Plants	Date	Mean temperature 9 a. m.–12 m.	Crawlers emerging in interval—				
				6–9 a. m.	9 a. m.–12 m.	12 m.–3 p. m.	3–6 p. m.	Total 6 a. m. to 6 p. m.
Number	*Number*		° *C.*	*Number*	*Number*	*Number*	*Number*	*Number*
47	6	Aug. 14	28.9	5	63	24	13	105
		Aug. 15	28.9	24	38	20	11	93
		Aug. 16	29.1	6	46	5	12	69
		Aug. 17	30.2	10	21	10	6	47
		Aug. 18	29.6	1	28	14	2	45
		Average		9.2	39.2	14.6	8.8	71.8
55	4	Aug. 21	30.4	24	42	9	9	84
		Aug. 22	29.5	12	52	11	11	86
		Aug. 23	29.4	1	35	1	13	50
		Aug. 24	28.7	4	56	10	5	75
		Aug. 25	28.8	17	37	3	5	62
		Aug. 26	29.4	16	21	6	3	46
		Average		12.3	40.5	6.7	7.7	67.2

The time of emergence depended in part upon the temperature. The warmer the day the earlier the crawlers tended to emerge. When the mean temperature between 9 a. m. and 12 m. was 25° C., 80 percent of the daily emergence occurred during that time, but when the mean temperature for this interval increased to 31° C., enough crawlers emerged before 9 a. m. to reduce the emergence between 9 a. m. and 12 m. to 40 percent of the daily total.

The effect of temperature upon the percentage of crawlers emerging between 11 a. m. and 2 p. m. each day in August 1923 and in March 1925 is shown in figure 4. During the summer, when the minimum temperatures were high, the total number of crawlers that emerged on any day depended largely upon the number that hatched on that or the preceding day, and not on the temperature. For August, therefore, the emergence between 11 a. m. and 2 p. m. has been calculated as a percentage of the number that emerged for the entire day. In the spring, however, temperatures were too low for crawlers to come out from the covering, as was shown by the greatly reduced emergence on cool days as compared with that on warm days. In March the daily emergence of crawlers from 39 mature females on 6 plants depended so largely upon temperature that emergence between 11 a. m. and 2 p. m. could not be referred satisfactorily to the emergence for the entire day, as in the summer. Instead, the daily totals were plotted against the date, and a smooth curve (not shown) was fitted to the records for those days when low temperatures did not retard emergence. This curve was principally a correction for the continuously decreasing production that has been noted previously in the paper. The number of crawlers emerging between 11 a. m. and 2 p. m. has been converted into a percentage of the number expected from the curve, and in figure 4 this percentage has been plotted against the average temperature of the interval.

It is shown that when midday temperatures fell below 20° C. very few crawlers emerged, either then or at any other time during the day. With midday temperatures of 23° C. and above, it is probable that temperature did not retard emergence.

External conditions that brought crawlers out onto the plant favored crawling. Of the individuals reported in table 6, few that emerged in the morning were settled at the time of examination, but

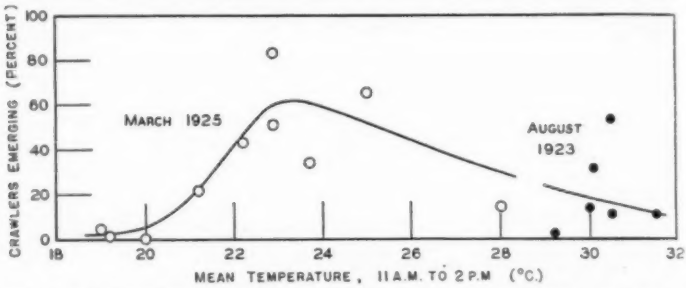

FIGURE 4.—Percentage of emergence of crawlers of *Pseudaonidia duplex* at midday (11 a. m. to 2 p. m.) as related to the temperature at this time, March 1925 (empty circles) and August 1923 (solid circles). The percentage emerging in March is based upon an estimated possible daily total; emergence in August is a percentage of the observed daily total.

late in the afternoon a fair proportion had settled in the 3-hour interval.

After emerging, the nymph generally crawled toward the light, a tendency that kept the population moving out onto the younger growth of the plant. During the winter of 1927 counts were made of

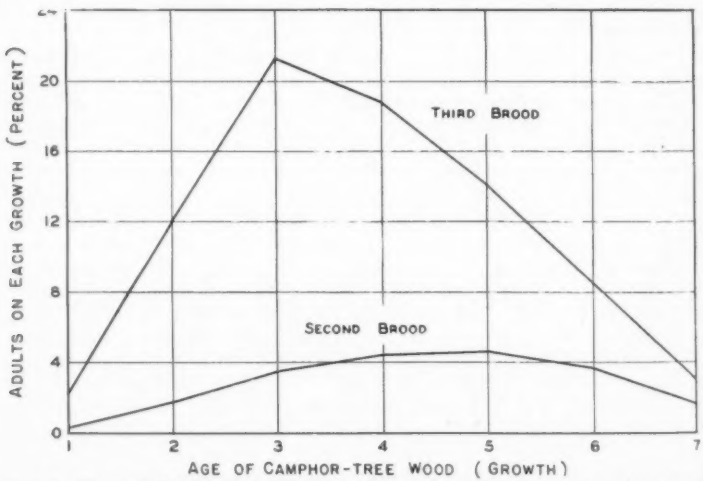

FIGURE 5.—Percentage distribution of adult females of the second and third broods on camphor-tree wood of different ages in the winter of 1927, showing the movement of the new brood toward the younger growth.

scales on camphor-tree wood of different ages, the counts of old spent females of the second brood being kept separately from those of overwintering females of the third brood that had not yet produced. The percentage occurrence of each type of female on wood of each growth is plotted in figure 5. This shows that the scales of the new generation, comprising 80 percent of the total, had moved

about one growth toward the newer wood. As there are normally three growths of camphor-tree wood and three generations of the camphor scale each year, this rate of migration should keep the population on the same growth throughout the year.

RATE OF CRAWLING

Because of its importance in the spread of the camphor scale, crawling was studied experimentally. Adult scales were scraped from infested twigs onto sheets of paper. Active uninjured nymphs crawled free of the debris toward the light and could then be transferred on the tip of a fine moistened brush to a potted plant.

An apparatus was set up in the constant-temperature room for investigating the accuracy of orientation and the rate of crawling in relation to light intensity and temperature. This apparatus consisted essentially of a sliding wooden stage on which a sheet of smooth, black, unglazed paper measuring 5½ by 7 inches was fastened, and a light source, usually a 75-watt mazda bulb, which supplied unilateral light filtered through a copper chloride solution at a level slightly above that of the stage. A crawler was placed on the sliding stage as far as possible from the light source, and the stage was moved back as fast as the insect crawled toward the light, so that it was illuminated uniformly throughout its trail. As it crawled, the larva was followed closely with a white pencil and its positions at 30-second intervals were indicated by cross marks. A mercury thermometer was suspended over the crawler and read at the beginning and end of each 5-minute trail. There was sometimes a difference as great as 1.3° C. between the temperature at the beginning and the end of a trail, because of the differential on which the thermostat operated. In such cases the temperature was interpolated for any given section of the trail.

To obtain as consistent a group of records as possible, the distance crawled in 1 minute without marked change in the angle of orientation was adopted as the unit, and as many 1-minute records as possible were selected from the many original records. Some crawlers gave five or more unit records; for others none were available. For each unit the angle of orientation from the light source, the most probable temperature (by interpolation), and the distance crawled were determined. The distance was measured by enlarging the trail five times with a pantograph, measuring the enlarged trail with a map measure, and converting back to centimeters of original trail.

The three typical trails reproduced in figure 6 illustrate the irregular orientation that was encountered, as well as the greater speed of crawling at the higher temperatures.

These experiments were started in June 1929 with second-brood crawlers and completed in September with third-brood crawlers. It was found that the crawlers did not behave uniformly. The majority of trails at a given temperature checked closely with one another, but a few nymphs crawled consistently much more slowly. These were designated as "slow" individuals and handled separately from the others. They crawled about one-half as fast and oriented themselves less accurately toward the light. They were more frequent toward the end of production by the second brood in September (20 percent of the total) than in June, perhaps because of the lower

vitality of crawlers late in the season. Some of them, however, may have been sluggish because they were isolated too soon after hatching, a condition that would not occur in the field. They will not be considered further.

The relation of temperature and light intensity to rate of crawling was tested over a temperature range of from 17° C. to 37° C. under a 75-watt lamp, a 15-watt lamp, and in early morning sunlight. A 75-watt light delivered to the crawlers a radiation intensity of 0.0029 to 0.0038 gram-calorie per square centimeter per minute. It was found that the observations at 75 watts could be adequately fitted with a straight line by the method of partial means (fig. 7), indicating that the percentage increase in rate of crawling per degree rise in temperature was constant over the entire range of temperature. This is in contrast to other activities of the camphor scale, such as oviposition and settling, which were frequently retarded by temperatures above 30° C. A reduction of 80 percent in light intensity (to approximately 0.007 gram-calorie per square centimeter per minute) by substituting a 15-watt bulb did not change the rate of crawling; therefore, these observations have been used with those under 75-watt illumination in determining the curve. The greater intensity of early morning sunlight, however, increased the rate of crawling materially.

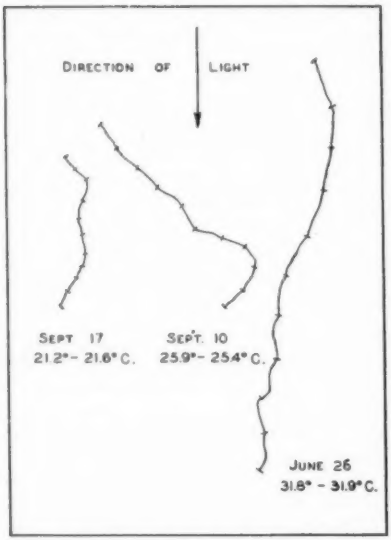

FIGURE 6.—Specimen trails of *Pseudaonidia duplex* crawlers under unilateral illumination of 75-watt bulb, showing type of orientation in the trail of a single crawler and the relative speed of crawling at different temperatures. The cross marks were made at 30-second intervals. One-fourth natural size.

Orientation was much less exact than had been expected, and the crawlers deviated as often to the right as to the left. In figure 6 it is shown that in the course of a 5-minute trail the crawler sometimes shifted both the angle and the general direction of orientation. Several crawlers described complete circles under unilateral illuminations. The angular departure from the direction of the light rays was greater at the lower than at the higher temperatures, averaging from 36° to 54° at 17° C. to 19° C. and from 13° to 18° at 33° C. to 36° C. A closer correlation between the angle of orientation and the rate of crawling is shown in figure 8, together with the number of 1-minute trails for each 5° of angular departure from the direction of light. It is shown that few nymphs crawled directly toward the light. The average angle of departure from precise orientation was 28.1°. As the orientation became less exact, the rate of crawling diminished by a constant percentage per degree of angular departure (upper curve in fig. 8).

Within the range of light intensities used in these experiments, an increase in light decreased the accuracy of orientation if it had any effect at all. The average angular departure from direct orientation toward the light was $23.1° \pm 1.3°$ at 15-watt illumination, $28.1° \pm 0.6°$ at 75-watt illumination, and $33.9° \pm 2.2°$ in early morning sunlight.

RATE OF SETTLING

The crawler is more exposed to death while it is active than after it has attached itself to the host by settling. Accordingly, the rate at which settling occurred has been studied in some detail. The ends of

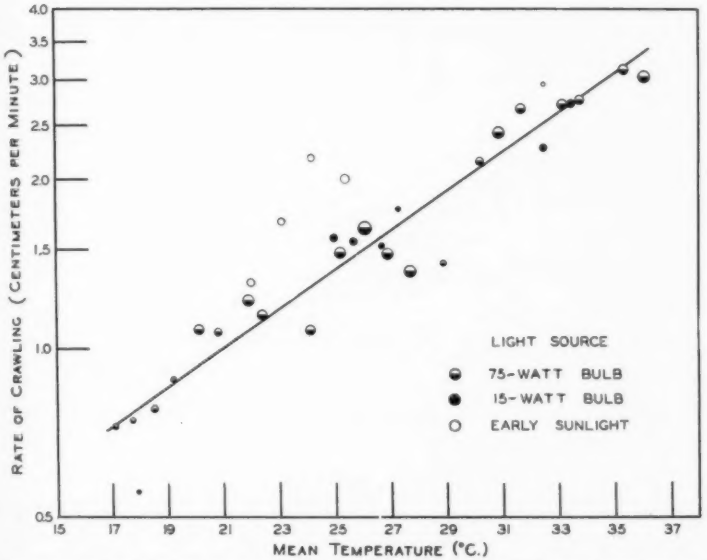

FIGURE 7.—Relation between rate of crawling of *Pseudaonidia duplex* nymphs and temperature under light intensities of a 75-watt lamp, a 15-watt lamp, and early morning sunlight, in a constant-temperature room, 1929. A logarithmic curve has been fitted to the 75-watt and 15-watt data. The diameter of each circle is proportional to the square root of the number of 1-minute trails upon which the reading is based.

short sections of second- or third-growth camphor-tree twigs, each with a single leaf, were sealed in shell vials containing water, and each twig was mapped and then infested with 20 crawlers. The position of each inactive crawler was noted hourly, and the time of settling was based upon the first observation at which the crawler was found in its final location. A few twigs (5 out of 25) on which the settling was abnormally low, presumably as a result of handling, were discarded. Two series of observations were carried out in 1926 on third-brood nymphs at midday before a large east window. The experiment was repeated on the first-brood crawlers in the spring of 1929 under artificial illumination from two 75-watt lamps in the constant-temperature room. The records are summarized in figure 9 and table 7.

TABLE 7.—*Rate of settling of Pseudaonidia duplex crawlers after being transferred to camphor-tree twigs in the laboratory*

Date	Temperature		Kind of light	Twigs	Crawlers	Total crawlers settling		Survivors settling—		
	Mean	Range						In 3 hours	In 6 hours	On stem
1926	°C.	°C.		*Number*	*Number*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
Sept. 23	30.2	1.9	Daylight	10	200	126	63.0	78.8	92.9	72.2
Sept. 28	32.2	2.5	do	10	200	86	43.0	89.4	98.8	37.2
1929										
Mar. 27	28.0	1.0	Two 75-watt lamps.	11	220	168	76.4	86.9	98.2	37.5
Mar. 28	27.5	1.0	do	12	240	163	67.9	78.5	90.2	46.3
Apr. 1	22.5	1.0	do	12	240	100	41.7	72.0	91.0	57.0
Apr. 2	22.5	1.0	do	12	240	92	38.3	70.7	94.6	52.2

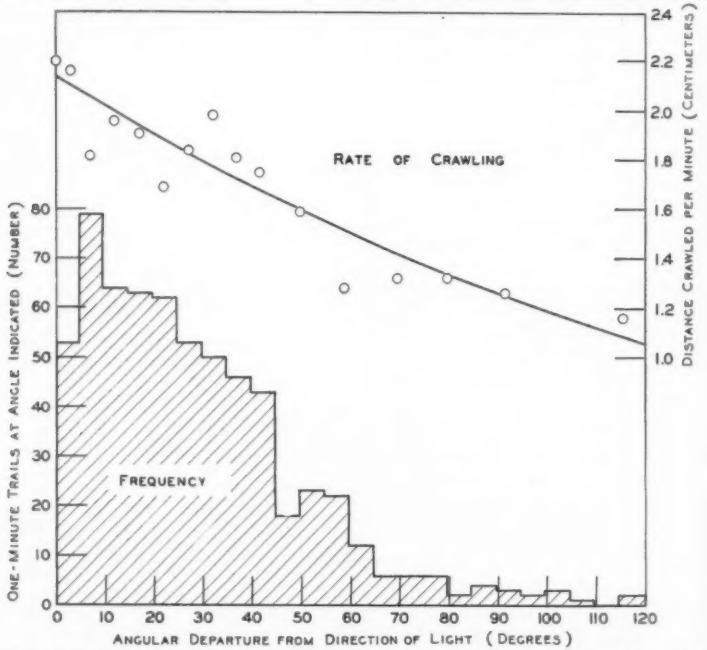

FIGURE 8.—Accuracy of orientation of *Pseudaonidia duplex* in crawling toward light from a 75-watt bulb as shown by the frequencies of angular departure and the rate of crawling at different angles.

In 1929 nearly twice as many crawlers settled at 27.5° C. as at 22.5° C., and those that did settle successfully did so more rapidly at the higher temperature. In the higher temperature range of the two experiments in daylight in 1926 fewer settled than at 27.5°, and though only two-thirds as many survived at 32.2° C. as at 30.2° C., the survivors settled more rapidly. Temperature changes in either direction from an optimum of 27° to 30° C. apparently reduced the number of survivors. Within the entire temperature range of 22° to 32° C., however, more than 90 percent of the crawlers

that survived settled in the first 6 hours after being transferred to the twigs.

Most of the males are found on the leaves and most of the females on the wood of the camphor-tree. The rates of settling in the two positions have been compared in order to determine any possible relationship to sex differences in the crawlers. At the end of 2 hours at 27.5° C. 46 percent had settled, divided about equally between the stem and the leaves and petioles. Of those that required more than 2 hours to settle, 71 percent were found on leaves and petioles. Although this seemed to indicate that crawlers on leaves, mainly

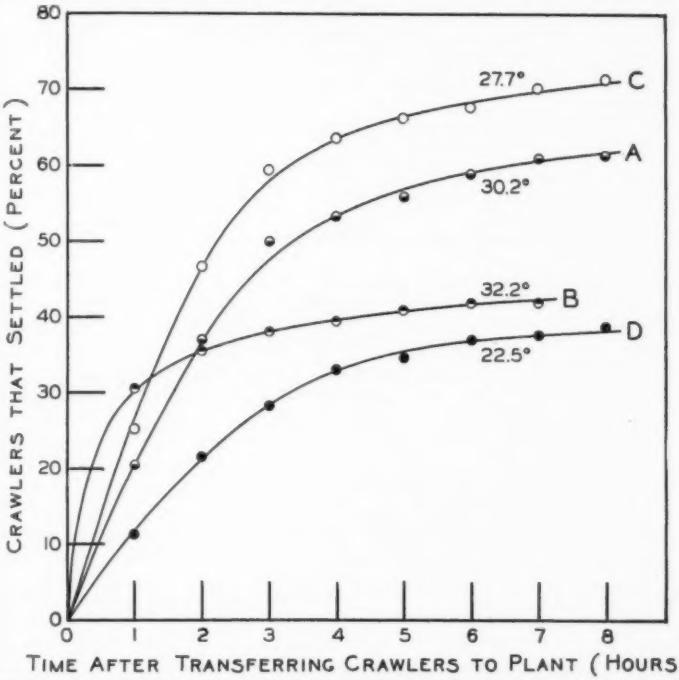

FIGURE 9.—Rate of settling of *Pseudaonidia duplex* crawlers on camphor-tree twigs and leaves: *A* and *B*, in daylight at 30.2° and 32.2° C., respectively, September 1926; *C* and *D*, under illumination of two 75-watt lamps, at 27.7° (mean for Mar. 27 and 28) and 22.5° (mean for Apr. 1 and 2), respectively, 1929.

males, required slightly longer to settle than those on stems, principally females, a comparison of the average time in the two positions showed no significant difference, being 2.37 ± 0.11 hours on the stem and 2.50 ± 0.08 hours on the leaves and petioles.

The positions of settling in the foregoing experiments have been classified in table 8. Of those on the stem, from 78 to 98 percent settled about nodes, bases of petioles, or similar irregular places. On the upper and lower surfaces of the leaves 86 to 100 percent settled next to the midrib and most of the remainder along large veins. The different parts of the leaf were favored as follows: 67 percent on the upper surface, 15 percent on the lower surface, and 18 percent on the petiole.

TABLE 8.—*Positions in which crawlers of Pseudaonidia duplex settled on small twigs, each with a single leaf, in the laboratory, 1926 and 1929*

Part of twig	Number of crawlers settling on date indicated							Total
	1926 [1]		1929 [2]					
	Sept. 23	Sept. 28	Mar. 26	Mar. 27	Mar. 28	Mar. 30	Apr. 1	
Stem:								
About nodes, etc.	89	29	68	55	63	46	50	400
Between nodes	2	3	19	10	8	5	7	54
Leaf:								
Upper surface								
Midrib	30	48	50	73	48	41	20	310
Blade	0	0	10	7	6	3	1	27
Lower surface:								
Midrib	0	2	24	10	11	7	10	64
Blade	0	0	2	2	0	3	2	9
Petiole:								
Top	5	3	9	13	10	5	5	50
Bottom	1	0	1	9	15	9	5	40

[1] In daylight.
[2] In constant-temperature room under artificial light, except on Mar. 26, when the observations were made in daylight.

FACTORS LIMITING THE NUMBER SETTLING

Many experiments were conducted in which crawlers were obtained from field-collected females, by methods already described, transferred by brush to potted plants, and the number that settled counted a day or two later. This method was subject to error from many causes, such as the premature transfer of crawlers, injury in handling, and abnormally adverse conditions on individual plants, peculiar to experimentation. In certain earlier experiments plants were omitted which seemed to fall in the last category, as judged by poor settling in comparison with the majority in the same group. Even with nearly ideal experimental conditions, the crawlers were affected by so many factors that the number that survived was unpredictable. As the experiments progressed, some of the more important conditions that reduced settling were disclosed, and this information was used wherever possible to improve the technic of the later experiments.

TEMPERATURE

The effect of temperature has already been shown in the experiments on rate of settling. The influence of temperature was, however, frequently masked by other factors. A series of experiments was therefore conducted under controlled conditions in which the other factors were kept as nearly constant as possible. On September 5, 1929, between 7:30 a. m. and 12:30 p. m., 100 crawlers from the same source were transferred to each of 30 plants and the plants were then placed in five chambers, each at a different temperature. The number that settled and formed complete covers at each temperature is shown in table 9 and figure 10. More crawlers settled at the higher temperatures, but the effect was not uniform with all the individuals. Apparently those settling on leaves were favored more by a rise in temperature than those on the stem. This tendency was observed also in the last column of table 7.

TABLE 9.—*Settling of crawlers of Pseudaonidia duplex on potted camphor-tree plants at different constant temperatures, morning of Sept. 5, 1929* [1]

Temperature (° C.)	Crawlers settled	First-instar nymphs on stem	Temperature (° C.)	Crawlers settled	First-instar nymphs on stem
	Percent	*Percent*		*Percent*	*Percent*
15.2	24.5±2.0	50.6±6.8	25.7	59.5±2.8	51.3±2.0
19.4	46.2±2.3	56.9±1.3	29.2	67.3±2.7	45.6±2.4
22.1	64.7±2.2	55.6±2.9			

[1] 100 crawlers on each of 6 plants at each temperature.

Settling records taken under outdoor temperatures in the shaded insectary or in the unheated laboratory also showed the effect of temperature. As these records were subject to variation in crawler

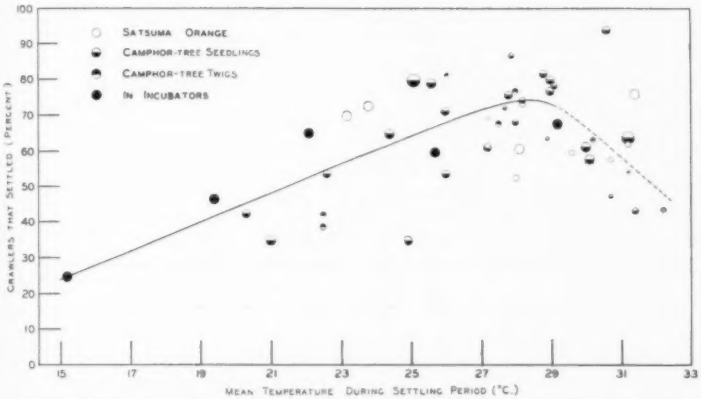

FIGURE 10.—Effect of temperature upon percentage of crawlers settling on different types of material. Original data in tables 7, 9, and 10. The diameters of the circles are proportional to the square root of the number of plants included in each mean.

material on different days, only crawlers transferred on a single day or half day were included in any given average, a procedure which increased both the validity of each mean and the variation between the means. The data have been summarized in table 10. The temperature was the average for the 6 hours following infestation when the exact time was recorded, an average for the period from 10 a. m. to 4 p. m. in the case of plants known to have been infested some time in the morning (1930), and an average from 10 a. m. to 8 p. m. when only the date was recorded. Most of the temperature records were taken from the insectary thermograph, and usually were sufficiently close to the temperatures of the plants, though occasionally, during rapid changes in outdoor temperature, there may have been a lag due to the absence of air currents about the plants. Beginning in 1926, plants were carefully protected from air currents during and after infestation, a procedure which undoubtedly increased the uniformity of settling.

TABLE 10.—*Settling of crawlers of Pseudaonidia duplex transferred to camphor-tree and Satsuma orange seedlings in shaded insectary or unheated laboratory*

CAMPHOR-TREE

Date	Time of day	Mean temperature	Plants	Total crawlers transferred	Crawlers settled	
1924:		°C.	*Number*	*Number*	*Number*	*Percent*
Apr. 8	p. m.	22.6	4	800	427	53.4
June 18		31.2	1	100	54	54.0
June 20		31.4	3	600	257	42.8
July 3		27.2	4	800	487	60.9
July 31		30.7	1	100	47	47.0
Aug. 18	p. m.	27.7	1	200	144	72.0
Aug. 19	a. m.	28.9	1	200	127	63.5
Do	p. m.	28.2	3	600	422	70.3
Aug. 20	a. m.	28.0	3	600	407	67.8
1925: Apr. 21		27.9	2	400	346	86.5
1926:						
Sept. 9	a. m.	31.2	11	900	571	63.4
Do	p. m.	30.1	7	600	344	57.3
Sept. 16	a. m.	29.1	3	450	351	78.0
Do	p. m.	27.8	4	600	452	75.3
1928:						
Aug. 30		26 ±	5	750	532	70.9
Sept. 8		29 ±	5	750	574	76.5
Sept. 14		29 ±	5	750	597	79.6
Sept. 18		26 ±	4	600	321	53.5
1929: Sept. 4	a. m.	30 ±	6	600	366	61.0
1930:						
Mar. 7	a. m.	20.3	5	1,500	629	41.9
Mar. 18	a. m.	24.9	6	1,800	622	34.6
Mar. 27	a. m.	21 ±	6	1,800	622	34.6
Apr. 30	a. m.	24.4	6	900	582	64.7
June 17	a. m.	30.6	5	750	702	93.6
Sept. 2	a. m.	28.8	4	600	488	81.3
Sept. 15	a. m.	25.1	12	1,800	1,432	79.6
Oct. 2	a. m.	25.6	6	900	709	78.8

SATSUMA ORANGE

1924:						
Apr. 8	a. m.	23.8	7	1,400	1,026	73.3
Do	p. m.	23.2	7	1,400	975	69.6
June 18		31.2	3	600	371	61.8
June 19	p. m.	27.2	3	600	368	61.3
June 20		31.4	8	1,600	1,211	75.7
June 24		29.6	2	400	238	59.5
July 3		27.2	1	200	138	69.0
July 31		30.7	2	200	115	57.5
Aug. 18	p. m.	28.0	3	600	314	52.3
Aug. 19	p. m.	28.2	3	600	438	73.0
Aug. 20	a. m.	28.1	8	1,600	966	[1] 60.4

[1] Unequal numbers on different plants equalized on basis of 100.

The relation between temperature and the percentage of crawlers that settled and formed covers, under controlled conditions on camphor-trees, and under sheltered outdoor temperatures on camphor-tree and Satsuma orange plants, has been plotted in figure 10. In the temperature range from 15° to 29° C. each degree centigrade increase in temperature tended to increase the number settling by about 3.5 to 4 percent, representing an average survival range of from 25 to 75 percent, but above this temperature there was usually a reduction in the number that settled. It has already been noted that during cool weather crawlers sometimes remained under the parent scale covering and delayed emergence until the midday temperature reached 20° C. If this occurs generally, settling in the field would seldom average below 40 percent of normal emergence. On the other hand, on very warm days the earlier morning emergence of crawlers might also increase survival.

Air Currents

Although most of the emerged crawlers were easily dislodged by the wind, more survived than had been anticipated. On September 16, 150 crawlers were placed on each of 13 plants in still air, and immediately every alternate plant was placed 24 inches from a 12-inch oscillating fan. These plants were thus exposed intermittently to a maximum air velocity of 12.7 miles per hour, or an average of 5.3 miles per hour, at temperatures from 28° to 30° C. On the other 7 plants, which remained in still air, 76.4 ± 1.3 percent survived, but on the 6 plants under the fan only 37.4 ± 0.9 percent survived. Of these, less than half could have inserted their beaks in the 20 to 35 minutes required to infest the plants in still air before they were placed before the fan. Hence, one-fourth to one-third of the active crawlers were able to settle in a fairly strong intermittent breeze. In the field, however, some of the crawlers dislodged by the wind were probably carried to other host plants.

Wind velocities equal to those under the fan were estimated (from Weather Bureau data) to occur, in the natural habitat of the camphor scale in New Orleans, on 1 day in 5 in April, but only on 1 day in 30 in July and August.

Light

The number of crawlers settling in the light or in moderate shade has been compared with the number settling in an adjacent paper dark box. On September 16, 1926, 150 crawlers were transferred to each of 13 potted camphor-trees, 7 of which were placed in the dark box immediately after being infested. A subsequent count showed that 76.4 ± 1.3 percent settled in the light, and only 64.8 ± 2.8 percent settled in the dark. The difference, 11.6 ± 3.1 percent, was significant.

The difference between males and females in the position of settling was somewhat less pronounced upon the camphor-trees in the dark box than upon adjacent plants in daylight. The percentage distributions of each sex upon leaf blades, leaf petioles, and stems in the light and in the dark are compared in table 11 for scales which developed to a stage in which the sexes could be separated. The experiment of 1926 was repeated in 1929 with this modification: Field infestations were scraped into small cones on the stem from which crawlers emerged and infested the plant over a period of 3 days, on some plants in daylight, on others in the dark. The duplicate plants for each treatment and sex were compared with one another by means of the χ^2 test to determine whether the proportions of crawlers which settled upon any given part of the plant were equal within the limits of chance. In both seasons parallel plants differed more from one another in two or more categories than could be attributed to chance, so that the significance of the different comparisons has been determined from the observed variability by the analysis of variance, as described by Fisher.[3]

[3] Fisher, R. A. Statistical methods for research workers. Ed. 2, pp. 178-237. Edinburgh and London. 1928.

TABLE 11.—*Comparison of position of settling of sexes of Pseudaonidia duplex in reference to light*

[In 1926 the crawlers were transferred by brush on Sept. 16, and the scales counted on Oct. 18–Nov. 15. In 1929 scale material was scratched from field infestations, and placed in a small cone on the stem from which crawlers emerged and infested the plants Sept. 2–6; scales were counted Oct. 23–24]

Year and sex	Condition	Plants	Scales	Crawlers that settled on—			Variance in 3 positions of settling	Degrees of freedom	Sum of squares	Mean square	Ln. of S. D.	z
				Leaf blades	Leaf petioles	Stem						
		Number	*Number*	*Per cent*	*Per cent*	*Per cent*						
1926:												
Male	Light	6	132	47.7	32.6	19.7	Light *v.* dark	2	204.96	102.48	2.31	0.68
	Dark	7	191	37.2	36.7	26.2	Experimental error	22	580.43	26.38	1.64	
Female	Light	6	150	2.7	4.7	92.6	Light *v.* dark	2	324.12	162.06	2.54	1.87
	Dark	7	141	14.9	5.0	80.1	Experimental error	22	83.64	3.80	.67	
1929:												
Male	Light	7	791	86.8	10.7	2.5	Light *v.* dark	2	256.48	128.24	2.43	1.17
	Dark	5	219	82.2	10.5	7.3	Experimental error	20	249.62	12.48	1.26	
Female	Light	7	916	21.0	3.2	75.8	Light *v.* dark	2	1055.08	527.54	3.13	.60
	Dark	5	302	26.8	5.3	67.9	Experimental error	20	3180.76	159.04	2.53	

The results show that of the males, which normally occur sparingly on the stem or wood of camphor-trees, a larger percentage settled on the stem in the dark than in the light in both experimental series. Of females, more settled on the leaves in the dark than in the light. This difference was not vitiated by the relatively heavier infestations of both sexes on the stems of the smaller plants used in 1926 than occurred on the larger plants in 1929. Despite the relatively large variability between plants, the absence of light modified the position of settling significantly. The chance that the distribution on leaf blades, leaf petioles, and stems in the dark differed from that in the light exceeded 100 to 1 for females in 1926 and males in 1929, and nearly equaled or exceeded 20 to 1 for the other two categories. Most of the variability between parallel plants in 1929 was due to two plants, one in the dark and the other in the light, both showing an abnormally large amount of settling on the stem. If these two records are omitted, the duplicate tests are more nearly in agreement (by the χ^2 test), and the effect of light is statistically significant for both sexes ($n_1=2$, $n_2=16$; male $z=0.77$, female $z=0.92$). From this we may conclude that a small part of the differential settling of the two sexes is clearly a visual response. An alternative explanation by differential mortality proved inapplicable.

SPECIES OF HOST

In 1924 the settling of crawlers on Satsuma orange was compared with that on camphor-trees. Table 10 shows 8 days or half-days when crawlers were placed on both camphor-tree and Satsuma orange plants. More crawlers survived on Satsuma orange than on camphor-trees in 6 of these cases, or an average of 6.4 percent more for the 8 cases. This difference was not statistically significant, indicating that Satsuma orange was no more susceptible to infestation than the camphor-tree. Many more female scales were found on Satsuma orange leaves than on camphor-tree leaves.

Time of Transference

Crawlers normally emerged from under the parent scale in the morning, but they could be obtained readily in the afternoon for infesting plants. On several days plants were infested both in the morning and in the afternoon with crawlers freshly scratched from the same field-infested material. (Table 12.) In 6 out of 7 cases

Table 12.—*Comparison of settling of crawlers of Pseudaonidia duplex in the morning and in the afternoon of the same day*

Date	Total crawlers transferred		Crawlers settled	
	In the morning	In the afternoon	In the morning	In the afternoon
1923:	*Number*	*Number*	*Percent*	*Percent*
June 11	1,175	750	38.6	34.1
June 12	1,250	1,500	42.6	38.7
June 13	1,700	1,500	58.2	48.1
1924:				
Apr. 8	1,400	1,400	73.3	69.6
Aug. 19	200	600	63.5	70.3
1926:				
Sept. 9	900	600	63.4	57.3
Sept. 16	450	600	78.0	75.3

the average percentage settling in the afternoon was smaller than in the morning of the same day. The average difference in these 6 cases amounted to about 5 percent of those transferred.

Crowding

The percentage that settled depended to a slight extent upon the number of crawlers transferred to the plant. The mean number settling and the standard deviation from the mean were compared when 50, 100, 200, and 300 were transferred on September 9, 1926. With only 50 nymphs the variation was excessive, but with 300 there seemed to be some loss from crowding on the 1-year-old seedlings used in this experiment.

SUMMARY

These studies deal with the productivity of the camphor scale and its biology from the time of egg deposition to the settling of the newly hatched nymphs, the period in which the maximum abundance of the succeeding generation is determined.

The productivity of the females in the laboratory was estimated in two ways, by counting the newly emerged scales on potted camphor-tree and Satsuma orange plants, and by removing the covering from females on camphor-tree twigs and observing oviposition directly. By the first method 13 first-brood females on camphor-trees produced an average of 125 offspring, and 24 second-brood females produced an average of 110. Productivity on Satsuma orange was somewhat higher. By the second method the females showed a higher total reproductivity than that estimated from emergence records. Between 14° and 29° C. the rate of oviposition increased with rising temperature according to logarithmic curves, but temperatures both below and above these limits retarded egg laying.

Productivity in the field was determined by counting the eggs beneath field-collected adults. The oviposition period ranged from 13 weeks for the overwintered females to 5 or 6 weeks for the first- and second-brood females.

The time required for the eggs to hatch varied widely with the age of the female. Eggs from the youngest females had the longest incubation period, but the older females retained their eggs longer after development had started. In most cases high temperatures shortened the embryonic period.

The crawlers showed a marked diurnal periodicity in the time of emerging from beneath the covering of the parent scale. They seldom came out between 6 p. m. and 6 a. m. The time of emergence during the day depended in part upon the temperature, being earlier on the warmer days. In midsummer the majority had emerged before 11 a. m. When midday temperatures fell below 20° C., few crawlers came out at any time during the day.

The crawlers were positively phototropic, but orientation toward a light source was not exact. The rate of crawling diminished as the angular departure from the light source increased, and was accelerated by high temperature within the range 17° to 37° C.

In experiments under controlled conditions the highest percentage of crawlers settled in the temperature range 27° to 30° C., but at all temperatures between 22° and 32° C. more than 90 percent of the crawlers that survived settled in the first 6 hours after being transferred to the twigs.

In insectary experiments each degree rise in temperature between 15.2° and 29.2° C. tended to increase the number of crawlers settling and forming covers by 3.5 to 4 percent. Since emergence is also suppressed by low temperatures, it is probable that settling in the field would seldom average below 40 percent of normal emergence.

The crawlers were easily dislodged by air currents. When infested plants were exposed intermittently to a maximum air velocity of 12.7 miles per hour, only 37 percent of the crawlers transferred settled, as compared with 76 percent on plants kept in still air.

Factors of lesser importance in their effect on crawler survival were type of host plant, light intensity, time of transfer, and number of crawlers per plant.

On camphor-trees most of the females settled on the stem and most of the males on the leaves. Of those on the stem, the majority settled around nodes and bases of petioles, while those on the leaves were usually found next to the midrib. The proportion of males and females settling on the leaf and stem was more nearly equal when settling took place in the dark, but the difference was not sufficient to explain the sex difference in location on the plant.

BIOLOGY OF THE CAMPHOR SCALE AND A METHOD FOR PREDICTING THE TIME OF APPEARANCE OF STAGES IN THE FIELD[1]

By A. W. CRESSMAN, *associate entomologist*, and C. I. BLISS, formerly *entomologist*, with the assistance of L. T. KESSELS, formerly *senior scientific aid*, and J. O. DUMESTRE, formerly *clerk, Division of Fruit Insects, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

In a previous paper the reproduction of the camphor scale (*Pseudaonidia duplex* Ckll.) and the behavior of the crawler stage were discussed (*1*).[2] The present paper is concerned with the later development of the insect, and includes the biology from the time of settling to the reproductive period, largely as determined from insectary experiments. It also deals with the seasonal history of the scale in the field and gives a method for predicting the time of appearance of each stage, which is useful in the timing of applications of insecticides so that they will be most effective.

METHODS USED IN BIOLOGICAL STUDIES

All the experiments were conducted in a screened insectary, where the scales were not exposed to direct sunlight or to rains. Potted camphor-tree plants 8 to 10 inches high were used as hosts in all the experiments on development. Material was taken from infested camphor-trees in the field. The scales, eggs, and crawlers were removed from the twigs to paper, and the crawlers transferred to the host plants with fine camel's-hair brushes. Only those nymphs that moved toward the light were used. During the transfer and settling of the crawlers the plants were shielded from air currents by glass frames.

Before a plant was infested, an ink line was drawn down the stem and a diagram made of the figure that would be formed if the stem were split on this line and the two halves, outer surface up, laid side by side, the leaves being drawn separately and numbered. Before the first molt the locations of the scales were indicated on this diagram and each was given a number. The individual scales could then be followed as long as they remained on the plant.

The number of insects that had formed covers at the end of 48 hours was taken as the number that had settled. Records of the length of each stage of the females were taken every day within about 2 hours of noon. In the case of the last three molts of the males usually 10 individuals were examined at 2-day intervals, but in cool weather the intervals between observations were sometimes longer. Daily mean temperatures were obtained by planimeter readings from thermograph records (checked against calibrated thermometers) on a 12-noon basis. Experiments were started with the first brood of the year and

[1] Received for publication Nov. 5, 1934; issued April, 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 283.

continued through the last brood in order to cover as wide a range of temperature as possible.

DESCRIPTION OF MOLTS

The female molts twice. Preceding each molt her body becomes swollen and its contents fluid, and the integument sticks to the covering. The time of molting can be detected by a color change in the covering. For several days previous to the molt this covering has a transparent amber color; then, as the molt takes place it takes on a

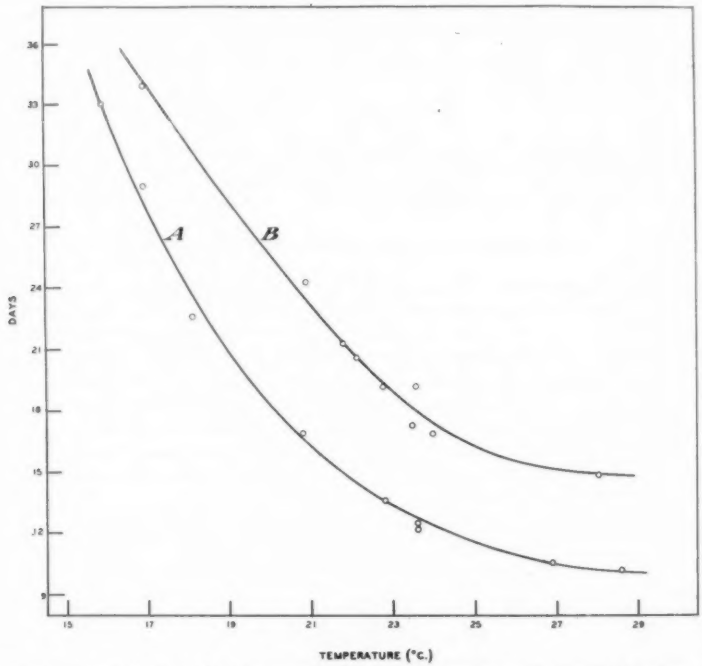

FIGURE 1.—Length of the first (*A*) and second (*B*) stadia of the camphor scale (*Pseudaonidia duplex*) as functions of the mean temperature.

drier, brighter appearance and loses its transparency. If the adult female is fertilized, the eggs are laid and hatched beneath the covering. The male is identical in appearance with the female when in the first larval instar, but in the second instar he becomes elongated and the covering is oblong-elliptical. The male has four larval instars. The fourth molt brings him into the winged adult stage, after which he emerges from beneath the covering and is ready to mate. The last three molts cannot be distinguished by color changes and the covering must be removed to ascertain the stage of the male beneath. The insect does not survive this treatment.

RATE OF DEVELOPMENT

The relation between the length of the first stadium (males and females) and temperature is shown in curve *A*, figure 1, where each point represents the mean for the scales on a group of plants infested at the same time. The length of this stadium was calculated from the time the scales were set out on the plant, since it had been previously shown (*1*) that most of the crawlers settle within a few hours after being transferred. The number of scales in each group ranged from 326 to 726, and there were 4,771 scales in all. The mean temperature

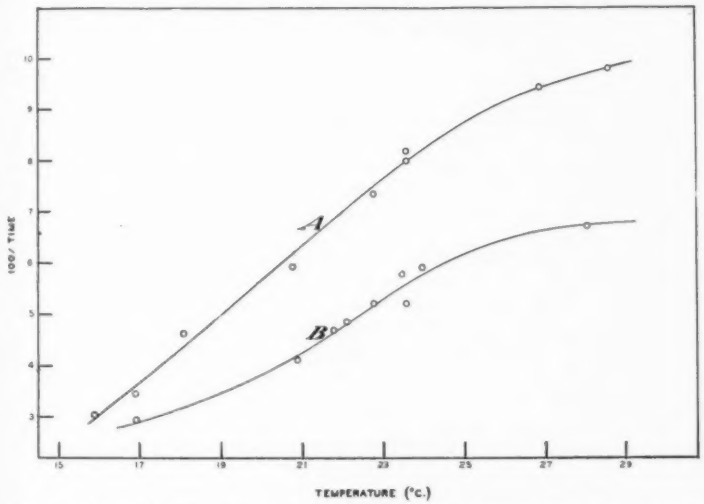

FIGURE 2.—The rate of development of the camphor scale in relation to temperature: *A*, During the first stadium; *B* during the second stadium.

ranged from 15.9° to 28.6° C. and the mean length of the stadium from 10.2 to 33 days. The numerical data are given in table 1.

TABLE 1.—*Length of the first stadium of camphor scales (both sexes) on potted camphor-tree plants in the insectary at different temperatures*

Brood	Date started	Scales	Average length of stadium	Mean temperature	Brood	Date started	Scales	Average length of stadium	Mean temperature
		Number	*Days*	*° C.*			*Number*	*Days*	*° C.*
First	Mar. 7	326	33.0±0.04	15.9	Second	June 17	726	10.2±0.01	28.6
	Mar. 18	456	29.1± .03	16.9	Third	Sept. 2	549	10.6± .02	26.9
	Mar. 27	439	22.6± .04	18.1		Sept. 15	582	12.5± .02	23.6
	Apr. 11	686	16.9± .02	20.8		Oct. 2	565	13.6± .02	22.8
	Apr. 30	442	12.2± .01	23.6					

The usual method of measuring relative growth velocities is by the reciprocal of the time required to complete a given stage. These values for different temperatures are shown in figure 2, *A*. The curve is approximately a straight line between 16° and 23.5° C., but beyond the higher point the effect of change in temperature on developmental

velocity decreased. Whether the decrease was due to the inclusion in the means of temperatures above 30° C. cannot be determined from these data.

A study of the statistical constants covering the relation of the length of the stadium to the temperature in these experiments showed that variables other than temperature had a negligible effect upon the rate of development. Curve *A* in figure 1 was fitted to the means by freehand graphic approximation, the algebraic sum of the residuals being made zero, and the difference between this curve and the observed individual record was read off for each case. The index of correlation was then calculated from the formula—

$$\rho_{yx}=\sqrt{1-\frac{\sigma^2_{y''}}{\sigma^2_y}\left(\frac{n-1}{n-m}\right)} \qquad (4)$$

This gave a value of 0.98, showing that the variation in length of the first stadium was almost entirely a function of temperature. Although the maximum time over which molting extended for any group was 7 days, the majority of scales molted closely around the mean date. In no case had as many as 10 percent molted 2 days previous to that time. No significant variation in the length of the stadium was found which depended on sex or position on the plant.

The results for the second-instar females are given in table 2 and figure 1, *B*. The second stadium is shown to be somewhat longer than the first at corresponding temperatures. In figure 2, *B*, the reciprocals of the time have been plotted against temperatures. No portion of this curve is a straight line. The index of correlation for 1,301 cases was 0.96. The maximum variation in length of the stadium for any group was 9 days, but again in no group did 10 percent of the scales show a 2-day variation from the mean.

TABLE 2.—*Length of second stadium of female camphor scales on potted camphor-tree plants in the insectary at different temperatures*

Brood	Date majority made first molt	Scales	Average length of stadium	Mean temperature	Brood	Date majority made first molt	Scales	Average length of stadium	Mean temperature
		Number	*Days*	*° C.*			*Number*	*Days*	*° C.*
First	Apr. 9	71	24.3±0.09	20.9	Second	June 27	259	14.9±0.04	28.0
	Apr. 16	171	21.3± .06	21.8	Third	Sept. 13	134	19.2± .04	23.6
	Apr. 19	113	20.6± .07	22.1		Sept. 27	138	19.2± .06	22.8
	Apr. 28	211	17.3± .04	23.5		Oct. 15	67	33.9± .06	16.9
	May 12	137	16.9± .03	24.0					

Variation in the length of both the first and second stadia was greatest in the scales of the first brood, as would be expected from the lower temperatures prevailing. Since in this brood the temperature at the time of the second molt was higher than at the time of the first molt, those scales that completed the first molt early were exposed to a lower mean temperature during the second stadium than those molting later. Consequently, there was an apparent tendency for scales making the first molt earlier or later than the average to compensate for this difference in the second stadium. When the length of the second stadium for the individual scales in the first two

groups was associated with the length of the first stadium, regression coefficients of −0.54 day and −0.53 day were obtained. When the differences in individual mean temperatures were considered, by correlating the difference between the expected length of the first stadium, predicted from figure 1, *A*, and the observed length with the equivalent departure in the second instar, the regression coefficient was not significant in one group, and in the second it had the small, though significant, value of −0.12 day. Although the first correlation was largely spurious, similar temperature conditions obtained in the field, and its existence in the case of the brood showing the greatest variation is further justification for using mean periods in field prediction. To use the minimum length of the stadium would introduce a systematic error, since those individuals in which the first stadium was shortest tended to spend a longer time in the next

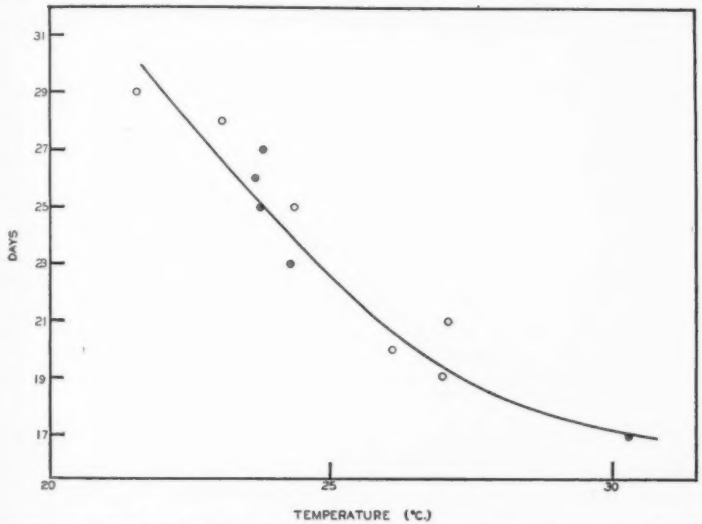

FIGURE 3.—Length of preoviposition period of the camphor scale in relation to temperature. Field data are shown by plain circles, insectary data by circles with crosses.

stadium than those that made the first molt at later dates. Peairs' (*6*) method of plotting minimum periods therefore has not been used.

The time from the second molt to the production of the first egg, or the preoviposition period, is shown in figure 3 and table 3. Because of the limited temperature range of the insectary studies, the data from the field counts have been included. Each entry represents a group of plants infested on the same day in the insectary studies or a brood in the field counts. Since it was necessary to depend on periodic removals for the insectary data, the results can be considered only as approximate. An additional source of variation lay in the fact that the exact date of fertilization was unknown. Therefore, the time the first females started to lay probably was the best indication of the end of the preoviposition period in the field, since these females were presumably the first to be fertilized. The data from both sources showed fair agreement.

TABLE 3.—*Length of preoviposition period of the camphor scale at different temperatures as estimated from field and insectary data*

Source of data	Mean temperature	Average length of period	Source of data	Mean temperature	Average length of period
	° *C.*	*Days*		° *C.*	*Days*
Field	21.6	29	Insectary	23.7	26
	23.1	28		23.8	25
	24.4	25		23.8	27
	26.1	20		24.3	23
	27.0	19		30.3	17
	27.1	21			

The time from the beginning of reproduction to the emergence of crawlers is shown in figure 4 and table 4. Because of the short

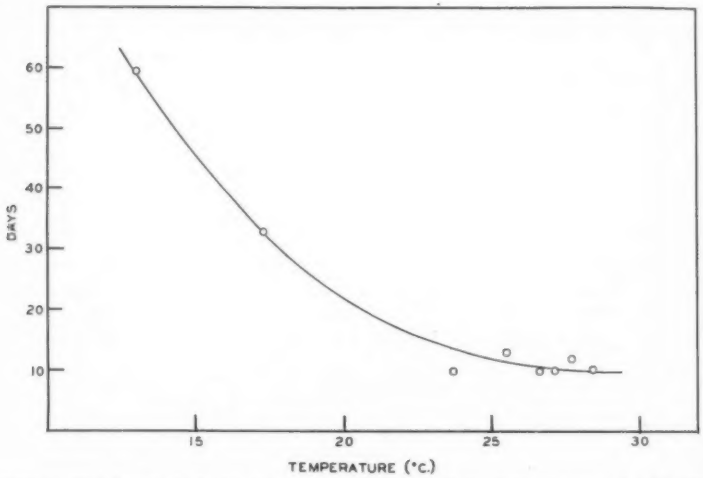

FIGURE 4.—Time from the beginning of egg production of the camphor scale to appearance of newly settled nymphs in relation to temperature. Each circle represents the period for a single brood as observed in the field.

interval between oviposition and hatching, and between hatching and emergence, the period from beginning of oviposition to emergence of young proved to be a more reliable interval than the two short periods. These records were taken entirely from the field counts.

TABLE 4.—*Period from appearance of first eggs to emergence of crawlers of the camphor scale at different temperatures, as estimated from field counts*

Mean temperature	Average length of period	Mean temperature	Average length of period
° *C.*	*Days*	° *C.*	*Days*
13.0	59	26.6	10
17.3	33	27.1	10
23.7	10	27.7	12
25.2	13	28.4	11

Males of the first instar showed no difference from the females. For the later stages periodic examinations were made as previously described. The number of days from the first molt to the time the majority had reached each succeeding stadium was determined, and the lengths of the stadia were calculated from these figures. Since only a small number were removed each time, and the last three stadia were of short duration, these results also were only approximate. The data are summarized in table 5.

TABLE 5.—*Length of later stadia of male camphor scales at different temperatures* [1]

Second stadium		Prepupal stadium		Pupal stadium		Adult (until emergence)	
Temperature	Length	Temperature	Length	Temperature	Length	Temperature	Length
° *C.*	*Days*	° *C.*	*Days*	° *C.*	*Days*	° *C.*	*Days*
21.0	25	19.7	3	15.9	15	13.6	3
21.9	22	21.7	3	23.2	5	22.6	5
22.2	20	23.0	3	23.4	4	23.5	3
22.8	19	23.7	3	23.9	5	23.8	1
23.6	18	24.0	3	24.1	5	24.1	3
23.6	18	24.9	2	24.5	6	24.4	4
24.0	16	25.2	2	24.8	4	25.0	3
28.1	16	26.2	2	26.6	3	27.0	3

[1] For length of the first stadium see table 1.

MORTALITY

The mortality for the first-instar females on the stem ranged from 5.9 to 33.1 percent, and for the second instar from 30.1 to 47.0 percent, and the percentage of adults that died without reproducing ranged from 23.8 to 47.6. If we consider the totals of all the experiments, the proportions that died during the three stadia calculated as percentages of those that entered the stadium were 11.0, 34.9, and 38.7 percent, respectively. The proportions of those settling that survived each stadium were 89.0, 56.4, and 33.4 percent, respectively. These figures have been corrected for scales killed by parasites and predators and those removed for observation. No relation between mortality and temperature or density of population was evident in these experiments. In the field, where the infestation is much heavier at times, population density is a factor, and subfreezing temperatures may kill some scales.

The mortality of the first-instar nymphs was somewhat higher on the leaves than on the stem. The removal of immature scales following the first molt, and the twisting and handling of the leaves during the examination, with the consequent loosening of the scale covers, made the figures on later mortality of doubtful value.

SEXUAL DIFFERENCES IN PLACE OF SETTLING AND RATIO OF SEXES

On camphor the two sexes show a marked difference in settling on different portions of the plant (table 6), the males generally going to the leaves and the females to the stem, although this characteristic has been observed to vary on different plants. Generally the males are found on the leaves, but the females on some plants settle on the leaves as readily as on the stem.

TABLE 6.—*Percentage of each sex of the camphor-scale nymphs settling on different parts of potted camphor-tree plants*

Sex	Total scales	Nymphs settling on—				
		Stem	Leaf blades		Petioles	
			Upper surface	Lower surface	Upper surface	Lower surface
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Female	3,050	75.8	4.6	12.6	3.1	3.9
Male	3,296	4.1	53.0	28.9	5.3	8.7

The sex was known only of those scales that survived well into the second stadium. On the basis of such scales, the proportion of males was 48.2 percent and of females 51.8 percent. Since the two sexes settled on different parts of the plant, these figures would not represent the true sex ratio if there was a difference in mortality before the sexes became distinguishable. It may be assumed, however, that the data on the scales of known sex do represent the true proportion of the sexes which settled on each part of the plant. If the total number that settled on each part is multiplied by the percentage of each sex later found, to give the true numbers of males and females in the newly settled nymphs, and the products are converted into percentages, we obtain males 51.9 percent, females 48.1 percent. The sex ratio, then, was practically unity.

FERTILIZATION

Fertilization is necessary for egg production. In a large series of experiments no parthenogenesis has been found. Unfertilized females never take on the purple-brown color characteristic of the gravid female. In cages 1 male has fertilized as many as 3 females, and it is possible that in the field the male can fertilize a still greater number. The female has also been observed to mate more than once.

SEASONAL HISTORY AND PREDICTION OF TIME OF APPEARANCE OF STAGES

METHODS OF PREDICTION

The two methods that have been used most by American entomologists for predicting the length of developmental stages are the "day-degree", or accumulated-temperature, method and the "developmental-unit" method.

The first method has been applied by Glenn (*5*) to data on the codling moth. It is based on the old idea that the time-temperature curve is an equilateral hyperbola, or that the developmental velocity-temperature curve is a straight line. Therefore, the product of time and effective temperature—i. e., degrees above the hyperbolic zero—will be a constant, so that when the number of day degrees required to complete the different stages has once been determined, it should

be possible to predict the length of the stages from the accumulated temperatures. It is doubtful whether the velocity-effective temperature curve is really a straight line over any part of the temperature range, but in many cases it is approximately so in the middle of the range. Glenn corrected for deviations at high, but not low, temperatures.

Shelford (*8*) discusses Glenn's work in some detail, and then develops a method that appears to be similar in principle although introducing more refinements. He uses the "developmental unit", which he defines as "the difference in amount of development produced in one hour by a difference of one degree of mean medial variable temperatures (other conditions being average)." "Medial temperature" refers to the straight-line (or approximately straight-line) portion of the velocity curve. This developmental unit, however, is a variable, the value of which depends on the climatic conditions, such as falling or rising temperatures or rainfall. Shelford makes corrections for high and low temperature, humidity, rainfall, etc. His use of hourly instead of daily temperature is a theoretical improvement, since there is less variation during the shorter period. On the whole, however, the method appears to be complicated and laborious as to both the fundamental experimentation necessary and the application to field prediction. Any method based on the assumption of a straight-line velocity curve requires so many corrections for accurate work that it is desirable to introduce a method that does not depend on such an assumption. For example, the velocity curve (fig. 2, *B*) for the second-instar females, as discussed on page 270, has no portion that is a straight line.

The method herein proposed is based on no assumptions whatever beyond that necessary to any prediction of this kind, that in the absence of other modifying external factors the development of an insect will repeat the same response to the same temperature. With the camphor scale a sufficient basis for prediction of the time of appearance of the different stages is the number of days required to complete a stage at any mean temperature, as shown in figures 1, 3, and 4. To get the expected date of appearance of any instar, a running average is kept of the mean daily temperatures until a temperature-time combination is reached which would permit completion of the stage.

This method may be illustrated with a prediction of the beginning of the second stadium of the first brood in 1926 (table 7). The first newly settled nymphs were observed on March 30. Beginning with this date, a running average was kept of the mean daily temperatures, and on the day (about Apr. 21) when this average approached a value that was indicated on curve *A* of figure 1, the number of days since March 30 was compared with the number of days corresponding to that temperature on the curve. This comparison was then repeated every day until the two periods were of the same length. The data given in table 7 indicate that the second instar should appear on April 25. The time of completion of the second stadium would then be calculated from this date and the readings made from figure 1, *B*.

TABLE 7.—*Data for prediction of date of appearance of the second instar of the first brood of camphor scales in 1926, when newly settled nymphs were first observed on Mar. 30, 1926*

Date	Length of period from Mar. 30	Average daily mean temperatures from Mar. 30	Length of first stadium as obtained from fig 1, *A*
	Days	° *C.*	*Days*
Apr. 21	22	16.8	29+
22	23	17.0	28
23	24	17.1	27+
24	25	17.3	26.5
25	[1] *26*	17.4	[1] *26*

[1] Agreement of numbers in italics indicates that the second instar may be expected to appear on Apr. 25.

The most probable source of error with this method lies in the averaging of temperatures on the basis of a time period instead of a fraction of a developmental period. If an insect spends 5 days at 28° C. and 5 days at 20° C., the mean temperature, calculated on a time basis, would be 24° C. But if the rate of development is twice as rapid at 28° as at 20° C., the portion of the stage passed at 28° would be twice as great as that at 20° C. This error, however, is less under a continuously varying temperature than where the insect is under one temperature for a certain period and is then suddenly changed to a very different temperature. The fact that the data on which the predictions are based were obtained under conditions similar to those in the field further reduces this error. The method is simple, direct of application, and should be capable of extension to many other insects.

SEASONAL-HISTORY DATA

Counts were made of scales on a row of camphor-trees in Audubon Park, New Orleans, at weekly intervals during 1926, 1927, and 1928. No thermograph records were kept, but the United States Weather Bureau maintains a substation in the park which records maximum and minimum temperatures. These temperatures were compared with the main Weather Bureau station extremes for New Orleans and the Weather Bureau means (computed on an hourly basis) modified accordingly to obtain the mean park temperatures. Since the maximum and minimum temperatures in the park were rather erratic as compared with the official temperatures, a constant rather than a day-to-day correction was applied. The mean park temperatures were consistently lower than the readings of the main Weather Bureau station, the average deviation for the period from the first appearance of newly settled nymphs to the maturing of the last brood being 1.0°, 0.9°, and 1.2° C., respectively, for the 3 years. Therefore, the daily mean temperatures given in the monthly summary of the Weather Bureau were reduced 1.0° C. for calculating the mean temperatures under which the scales developed. This illustrates the necessity of checking habitat temperatures of an insect against Weather Bureau records in order to allow for consistent departures.

Scales were recorded according to instars, and the adults were further divided into white, pink, gravid, and reproducing. The

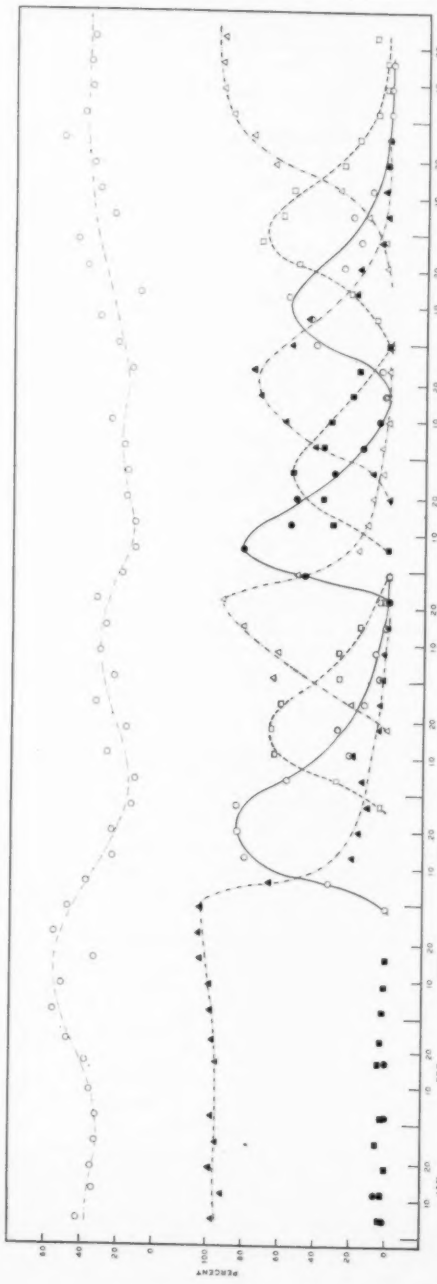

FIGURE 5.—The lower curves show the succession of stages of the camphor scales over an 11-month period in 1926. Circles represent the first instar, squares the second instar, and triangles the third instar. Outline and solid figures distinguish between successive generations. The ordinates are the percentages of the total living population in each stage. The upper curve shows the mortality over the same period, ordinates being the percentages of the total count that are dead.

numbers of living and dead individuals of each stage were tallied, with the dead separated into those killed by parasites, predators, freezing, and other natural causes. In addition, the number of eggs and crawlers under each reproducing female was recorded. Usually one twig was taken from each of four trees, and approximately 500 scales were counted from these twigs. Only the female scales are discussed here, the assumption being made that all the first instar and early second instar were females. As is seen from the proportion of sexes settling on the stem in the insectary, the error introduced is small.

When the beginning of a stage fell between the weekly counts, it was necessary to extrapolate the curves to the origin. Such curves were compared with those for other years to determine their most probable position. Except in the case of the second- and third-brood adults, the broods were separated with little difficulty. There was some question as to the time the third-brood adults first appeared, although when they were present in any numbers they were easily distinguished.

A graph on which the first year's counts are recorded is shown in figure 5. This is typical of the data for the other years, which were plotted with smoothed curves in the same way. As would be expected, the curves have a tendency to flatten in successive generations, and the tails on the curves are most pronounced in the third generation.

These counts present a picture of the scale population throughout the year. Their most important use is for checking the applicability of insectary data for prediction of the succession of stages in the field. They also show the incidence of parasites and, to some extent, of predators, and the effect of climatic conditions on mortality.

PREDICTION OF STAGES

In predicting the time of appearance of the different stages, the writers have worked with the time from the first of one stadium to the first of the next, for to figure from peak to peak would require that the two stadia involved be of the same length. They have started with the appearance of newly settled nymphs in the spring and checked the expected date of the appearance of each stage, calculated as described in the example given above, against the observed appearance of the stage in the field.

A comparison of the expected and observed dates for the 3 years is shown in table 8. On the whole the agreement is close, justifying the method. There are two serious deviations. One is a difference of 5 days for the first brood, second instar, in 1927. The fact that the expected dates tend to run later than those observed for this year indicates that the first newly settled nymphs in the field were missed in the counts. The second serious discrepancy is for the third brood, third instar, in 1926. Little importance can be attached to agreement or disagreement between observation and calculation at this point, because of the difficulty of ascertaining the actual beginning of this stage. As to the other discrepancies, a difference of a day is well within the variation of the material, and since counts were made at weekly intervals, the extrapolation sometimes necessary may be in error by a day or so.

TABLE 8.—*Comparison between observed and predicted dates of appearance of different stages of the camphor scale in the field*

Brood and stage	1926			1927			1928		
	Expected date	Observed date	Temperature	Expected date	Observed date	Temperature	Expected date	Observed date	Temperature
			° *C.*			° *C.*			° *C.*
First brood:									
First instar		Mar. 30			Feb. 21			Mar. 19	
Second instar	Apr. 25	Apr. 26	17.4	Mar. 27	Mar. 22	15.7	Apr. 16	Apr. 19	16.8
Third instar [1]	May 19	May 18	20.7	Apr. 17	Apr. 16	21.8	May 12	May 9	19.8
Second brood:									
Eggs	June 12	June 12	24.6	May 17	May 15	21.7	June 6	June 6	23.4
First instar	June 21	June 22	27.2	May 29	May 26	24.6	June 18	June 20	25.2
Second instar	July 3	July 5	26.8	June 9	June 7	26.6	June 29	June 29	26.5
Third instar	July 18	July 19	26.9	June 24	June 21	26.0	July 14	July 16	27.3
Third brood:									
Eggs	Aug. 7	Aug. 8	26.8	July 13	July 11	27.1	Aug. 4	Aug. 6	26.2
First instar	Aug. 17	Aug. 17	26.8	July 23	July 23	27.8	Aug. 14	Aug. 16	28.2
Second instar	Aug. 27	Aug. 27	27.6	Aug. 3	Aug. 3	26.4	Aug. 24	Aug. 27	27.9
Third instar	Sept. 11	Sept. 16	27.3	Aug. 18	Aug. 19	27.1	Sept. 8	Sept. 11	25.8

[1] The third-instar females are adults.

The agreement between the expected and observed results, therefore, is well within the accuracy of the data. To facilitate prediction

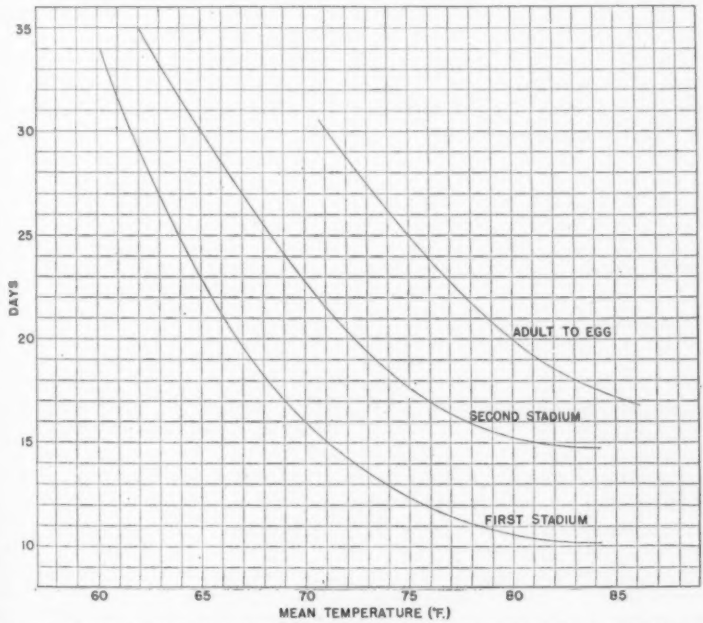

FIGURE 6.—Length of first and second stadia of nymphs and of preoviposition period of adults of the camphor scale as functions of the mean tempreature. Curves of figures 1 and 3 converted to Fahrenheit scale for use in prediction directly from Weather Bureau records.

directly from Weather Bureau records, the curves of figures 1, 3, and 4 have been reproduced in figures 6 and 7 with the temperature on the Fahrenheit scale.

In the preceding discussion the prediction of the appearance of eggs laid by overwintering females or of the first instar of the first brood from the temperature records has not been considered. If this could be done, there would be no necessity for making field observations, and prediction could be made entirely from the weather records.

An attempt was made to predict the appearance of eggs on the basis of mean temperature from an arbitrary date (Dec. 1) to the beginning of oviposition in the winter, based on seasonal-history data over the 5 years 1924–28. The points for 4 years fell on a smooth curve, but the date for 1924 was consistently earlier whether the point selected was first increase in production, 2, 5, or 10 eggs per female. That the reactions of the overwintering females differ from those of the preceding brood is shown by the fact that, if this brood gave the same response

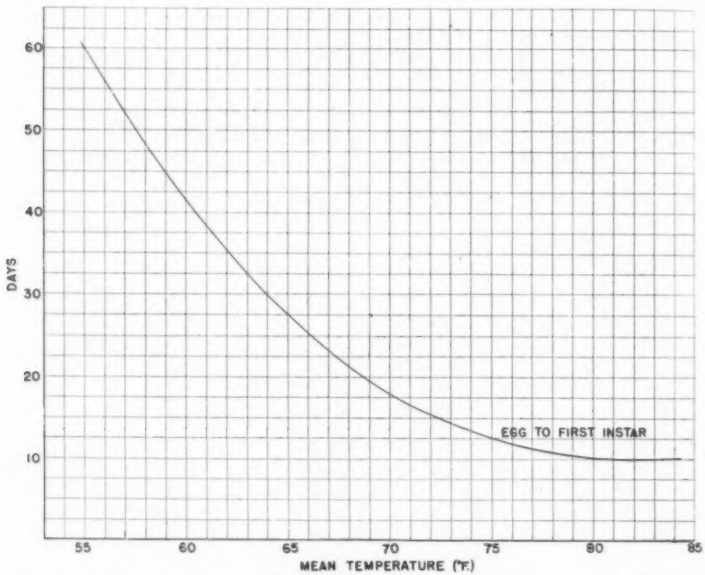

FIGURE 7.—Length of period from beginning of egg production to appearance of newly settled nymphs as a function of temperature. Curve of figure 4 converted to Fahrenheit scale for use in prediction from Weather Bureau records.

to temperature as the others in the 3 years (1926, 1927, and 1928) in which complete counts are available, eggs of another brood would have been produced each fall, whereas only in 1927 was there a partial fourth brood. In 1925, when the first adults of the third brood appeared about August 22, there were also a small number that reproduced. In both cases production, instead of gradually increasing, showed a tendency to become stationary and then decline during December and the first part of January. In 1923, 1924, 1926, and 1928 no fourth-brood nymphs were found. It is, therefore, probably not justifiable to select an arbitrary date, since oviposition in winter depends in part on the time of maturation the preceding fall. This is

shown by the following data from insectary experiments in which the second molt was completed at different times:

Date majority made second molt:	*Date of first eggs*
Oct. 2	Feb. 12
Oct. 16	Feb. 20
Nov. 18	Apr. 18

Complete temperature records on the plants involved are not available, but all were subject to the same conditions.

Until the reactions of the overwintering females are better understood, therefore, it is necessary to base predictions on the beginning of some nonoverwintering stage. The appearance of newly settled nymphs is preferable to the beginning of egg production, for several reasons. Since a few eggs are present throughout the winter, the date of the first continued increase in egg production is more uncertain than the appearance of newly settled nymphs. Furthermore, to determine the beginning of increased egg production may require counts over an extended period, while the newly settled nymphs are easily recognized and the date of their appearance can be ascertained with little difficulty.

FACTORS AFFECTING MORTALITY

The interpretation of the mortality figures is obscured by the fact that the scales often remained on the twig for some time after death, and the time of death was therefore unknown. In addition, since young were being produced during a large part of the time, successive counts were samples of a population whose make-up was constantly changing. For these reasons the figures represent only the portion of the population dead at the time of counting, and thus cannot be used to ascertain the proportion of those entering a given stage which failed to survive.

The mortality in 1926 is shown in the upper curve in figure 5 where, to permit comparison with the living scales, the same abscissas have been used in both cases. The mortality tended to reach a maximum just before the start of the new brood and a minimum at about the peak of the first instar. At these times the scales had been exposed to the maximum and minimum chances of death, since the average age of the scale population would be greatest at the first point, and least at the second.

The percentage dead in the overwintered brood was lower in 1928 than in 1926 or 1927, as a result of decreased parasitization; otherwise, there was little difference in the 3 years.

During the time in which mortality records were taken, there were 4 years in which the temperature fell below −5° C. In January 1924 minimum temperatures of −3.3° C., −7.2° C., and −2.8° C., respectively, on 3 successive days killed about 16 percent of the scales (*2*). In December 1925, when the temperature fell to −5.6° C., approximately 4 to 5 percent of the population were frozen. About the same mortality was experienced in January 1928 with minimum temperatures of −5.6° C. and −6.1° C. On February 8 and 9, 1933, when temperatures of −3.9° C. and −6.7° C. were recorded, between 30 and 37 percent of the scales on uninjured wood were frozen (*3*). In reality the total mortality in 1933 was much higher because most of the younger wood of camphor-trees was killed

back, and this wood bore most of the living scales previous to the freeze.

These records show considerable variation in the mortality resulting from comparable temperatures, the probable reasons for which have been found in examination of the seasonal-history records and weather records preceding the low temperatures. In 1924 and 1933 the months preceding the minimum temperatures showed a daily average excess of 7.9° and 5.8° C., respectively. In 1925 and 1927 the preceding months showed a daily average deficiency of 3.3° and 1.5° C., respectively. In the 2 years when subfreezing temperatures were preceded by subnormal temperatures, there was a much lower mortality than in those years when freezes were preceded by temperatures well above the normal. In all years except 1933 the scales were in the adult stage but had not started oviposition when the subfreezing temperatures occurred. In 1933 nearly 95 percent of the scales were reproducing, and the highest mortality was experienced, although the temperature did not go so low as in 1924. The records indicate that subfreezing temperatures are fatal to more scales when they have been preceded by warm weather than when they follow a period of subnormal temperature, and have greater effect the more advanced the development of the scale population.

The number of scales parasitized never assumed significant proportions except in the third broods of 1926 and 1927 and the over-wintered brood of 1926 (third brood of 1925). In these cases the parasitization was approximately 20 percent. This decreased rapidly with the emergence of the first brood, and during the spring and summer months seldom were more than 3 percent of the scales parasitized. That the increases in the third brood were not merely the result of the parasitized scales from the preceding broods remaining on the plant is indicated by the fact that in the second brood no increase over the first brood was noted, and that in 1928 the parasitization of the third brood was comparable to that in the earlier broods of that year.

The scales were usually killed in the second instar, but occasionally parasitized adults were found. The following parasites have been recovered: *Aphelinus fuscipennis* Howard, *Aspidiotiphagus citrinus* (Crawford), *Prospaltella aurantii* Howard, *P. fasciata* Malenotti, and *Signiphora flavopalliata* Ashmead. *Metalaptus torquatus* Malenotti has also been taken from cages, but this may be an egg parasite of Psocidae.

From these counts little can be said about the efficiency of predators, since the scale is usually completely removed when attacked. A bagworm, *Platoeceticus gloveri* (Packard), has been locally quite effective, and trees have been observed that were practically cleaned of scales by this insect (7). Two species of lady beetle, *Chilocorus bivulnerus* Mulsant and *Microweisea misella* Leconte, also feed on the scale, but none of these natural enemies has been sufficiently effective to be relied upon as a means of control.

SUMMARY

The biology of the camphor scale (*Pseudaonidia duplex* Ckll.) from the time of settling to the beginning of the reproductive period has been studied in the insectary and field.

The rate of development was found to be dependent largely upon the mean temperature, the index of correlation between the length of the first stadium and the temperature being 0.98, and for the length of the second stadium, 0.96. Curves are given for the length of the first and second stadia, the period from the second molt to egg production, and the time from the first egg to appearance of newly settled nymphs, as functions of temperature.

Of the females that settled on the stem, 88.9 percent completed the first stadium, 56.4 percent the second stadium, and 33.4 percent reproduced.

Approximately equal numbers of males and females were produced, and fertilization was necessary to reproduction.

The mean temperature from the date of emergence of the first brood was found to be a satisfactory basis for predicting the time of appearance of the different stages under field conditions, the calculated and observed dates for field counts extending over 3 years agreeing within the accuracy of the data.

As many as 30 to 37 percent of the scales have been killed by low winter temperatures. Resistance to subfreezing temperatures was greatest when they were preceded by subnormal temperatures and when the scales were in the earlier stages of development.

Parasites and predators have not been important factors in the control of this insect.

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